Integrating the Neurobiology of Schizophrenia

EDITED BY

ANISSA ABI-DARGHAM
OLIVIER GUILLIN

International Review of Neurobiology, Volume 78

International
REVIEW OF
Neurobiology
Volume 78

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ANISSA ABI-DARGHAM
Department of Psychiatry, Columbia
College of Physicians and Surgeons
New York State Psychiatric Institute
Columbia University, New York, USA

OLIVIER GUILLIN
Unité de Psychiatrie
CHU ch. Nicolle
Rouen, France

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Academic Press is an imprint of Elsevier

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525 B Street, Suite 1900, San Diego, California 92101-4495, USA
84 Theobald's Road, London WC1X 8RR, UK

This book is printed on acid-free paper. 

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ISBN-13: 978-0-12-373737-3
ISBN-10: 0-12-373737-0

PRINTED IN THE UNITED STATES OF AMERICA
07 08 09 10 9 8 7 6 5 4 3 2 1

CONTENTS

Deciphering the Disease Process of Schizophrenia: The Contribution of Cortical GABA Neurons

David A. Lewis and Takanori Hashimoto

Alterations of Serotonin Transmission in Schizophrenia

Anissa Abi-Dargham

Serotonin and Dopamine Interactions in Rodents and Primates: Implications for Psychosis and Antipsychotic Drug Development

Gerard J. Marek

Cholinergic Circuits and Signaling in the Pathophysiology of Schizophrenia

Joshua A. Berman, David A. Talmage, and Lorna W. Role

Schizophrenia and the $\alpha 7$ Nicotinic Acetylcholine Receptor

Laura F. Martin and Robert Freedman

Histamine and Schizophrenia

Jean-Michel Arrang

Cannabinoids and Psychosis

Involvement of Neuropeptide Systems in Schizophrenia: Human Studies

Brain-Derived Neurotrophic Factor in Schizophrenia and Its Relation with Dopamine

Schizophrenia Susceptibility Genes: In Search of a Molecular Logic and Novel Drug Targets for a Devastating Disorder

Joseph A. Gogos

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CONTRIBUTORS

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

Anissa Abi-Dargham (1, 133), Department of Psychiatry, Columbia College of Physicians and Surgeons, New York State Psychiatric Institute, Columbia University, New York 10032, USA; Department of Radiology, Columbia College of Physicians and Surgeons, New York State Psychiatric Institute, Columbia University, New York 10032, USA

Jean-Michel Arrang (247), INSERM, U573, Unité de Neurobiologie et Pharmacologie Moléculaire Centre Paul Broca, 2 ter rue d'Alésia, 75014 Paris, France

Joshua A. Berman (193), Department of Psychiatry, Columbia University College of Physicians and Surgeons, New York State Psychiatric Institute, New York, New York 10032, USA

Ricardo Cáceda (327), Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, Atlanta, Georgia 30322, USA

Deepak Cyril D'Souza (289), Department of Psychiatry, Yale University School of Medicine, VA Connecticut Healthcare System, West-Haven, Connecticut 06516, USA

Caroline Demily (377), Unité de Psychiatrie, UFR de Médecine et de Pharmacie de Rouen, France; CHU Ch. Nicolle et Centre Hospitalier du Rouvray, INSERM U614, UFR de Médecine et de Pharmacie de Rouen, France

Robert Freedman (225), Research Service, VA Eastern Colorado Health Care System, Denver, Colorado 80220, USA; Department of Psychiatry, University of Colorado at Denver Health Sciences Center, Denver, Colorado 80262, USA

Joseph A. Gogos (397), Department of Physiology and Cellular Biophysics, and Center for Neurobiology and Behavior, Columbia University, College of Physicians and Surgeons, New York, New York 10032, USA

Yukiori Goto (41), Department of Neuroscience, University of Pittsburgh, Pittsburgh, Pennsylvania 15260, USA; Department of Psychology, University of Pittsburgh, Pittsburgh, Pennsylvania 15260, USA; Department of Psychiatry, University of Pittsburgh, Pittsburgh, Pennsylvania 15260, USA

Anthony A. Grace (41), Department of Neuroscience, University of Pittsburgh, Pittsburgh, Pennsylvania 15260, USA; Department of Psychology, University of Pittsburgh, Pittsburgh, Pennsylvania 15260, USA; Department of Psychiatry, University of Pittsburgh, Pittsburgh, Pennsylvania 15260, USA

Olivier Guillin (1, 377), Department of Psychiatry, Columbia College of Physicians and Surgeons, New York State Psychiatric Institute, Columbia University, New York 10032, USA; Unité de Psychiatrie, UFR de Médicine et de Pharmacie de Rouen, France

Takanori Hashimoto (109), Department of Psychiatry, University of Pittsburgh, Pennsylvania 15213, USA

Daniel C. Javitt (69), Program in Cognitive Neuroscience and Schizophrenia, Nathan Kline Institute for Psychiatric Research, New York University School of Medicine, Orangeburg, New York 10962, USA

Becky Kinkead (327), Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, Atlanta, Georgia 30322, USA

Marc Laruelle (1), Department of Psychiatry, Columbia College of Physicians and Surgeons, New York State Psychiatric Institute, Columbia University, New York 10032, USA; Department of Radiology, Columbia College of Physicians and Surgeons, New York State Psychiatric Institute, Columbia University, New York 10032, USA

David A. Lewis (109), Department of Psychiatry, University of Pittsburgh, Pennsylvania 15213, USA; Department of Neuroscience, University of Pittsburgh, Pennsylvania 15213, USA

Gerard J. Marek (165), Eli Lily and Company, Lilly Research Laboratories, Psychiatric Disorders Discovery Biology, Lilly Corporate Center, Indianapolis, Indiana 46285, USA

Laura F. Martin (225), Research Service, VA Eastern Colorado Health Care System, Denver, Colorado 80220, USA; Department of Psychiatry, University of Colorado at Denver Health Sciences Center, Denver, Colorado 80262, USA

Charles B. Nemeroff (327), Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, Atlanta, Georgia 30322, USA

Lorna W. Role (193), Department of Psychiatry, Columbia University College of Physicians and Surgeons, New York State Psychiatric Institute, New York, New York 10032, USA; Department of Cell Biology in Pathology, Columbia University College of Physicians and Surgeons, New York, New York 10032, USA

David A. Talmage (193), Institute of Human Nutrition, Columbia University College of Physicians and Surgeons, New York, New York 10032, USA

Florence Thibaut (377), Unité de Psychiatrie, UFR de Médecine et de Pharmacie de Rouen, France; CHU Ch. Nicolle et Centre Hospitalier du Rouvray, INSERM U614, UFR de Médecine et de Pharmacie de Rouen, France

PREFACE

"Integrating the Neurobiology of Schizophrenia" is meant to bring together the current knowledge implicating various neurotransmitter systems in the disease of schizophrenia while placing a big emphasis on their interactions. The goal is to build through each chapter one of the blocks leading to an integrative model showing how one neurochemical alteration could contribute to the final common pathway of another neurochemical dysregulation observed in this illness. It is intended to be a reference for clinicians, scientists, and students who want to learn more about the different neurotransmitters that may play a role in schizophrenia.

To anchor these discussions, we adopt the view that all neurotransmitter alterations may lead to the dopaminergic alterations observed in this illness. This point of view is an arbitrary oversimplification, but complex problems may be better addressed once broken down into simple questions. This view is suggested by the observation that alterations in dopamine transmission are most directly linked to the symptoms of the illness as well as the response of these symptoms to antipsychotic treatment. Recent research, mostly from imaging, over the last decade, has greatly advanced the field by providing strong evidence for few fundamental observations: studies have shown definitively that subcortical D2 hyperstimulation is associated with positive symptoms and more recent data has linked negative symptoms and cognitive disturbances with cortical dopamine dysfunction. Studies have also shown conclusively that all effective antipsychotics show significant D2 receptor occupancy. Despite the fact that there is no clear relationship between occupancy and clinical response, and there is no clear definition of the minimal occupancy needed to achieve therapeutic efficacy, this remains the most solid finding in antipsychotic therapy. We previously reported that elevated levels of striatal intrasynaptic DA concentration is predictive of fast response to antipsychotic drugs in patients with schizophrenia (Abi-Dargham *et al.*, 2000). Thus, the extent of the therapeutic response to D2 receptor antagonism is affected by the underlying pathology. Patients whose pathology is associated with excessive stimulation of D2 receptors by DA respond well to D2 receptor blockade. Conversely, patients who present a psychotic episode in the absence of detectable changes in synaptic DA levels are poor responders to current antipsychotic drugs. On the other hand, we did not observe a relationship between subcortical D2 hyperstimulation and the response of negative symptoms to treatment at 6 weeks. Overall these findings suggest that D2

hyperstimulation is relevant to the treatment of positive symptoms in most but not all patients with schizophrenia and is probably irrelevant to negative symptoms. This highlights the complexity of the problem. A provocative study by Seeman *et al.* (2006) has provided some support to the concept of common final pathway through hyperstimulation of D2 receptors leading to psychosis, essentially by increasing the high affinity states of dopamine D2 receptors.

In this book, we first present a comprehensive review of the alterations in dopamine (see the chapter by Guillin, Abi-Dargham, and Laruelle) and the underlying cellular and physiological events that may accompany them (see the chapter by Goto and Grace). Then we attempt to review most of the neurobiological alterations that may be implicated in schizophrenia and may contribute to the symptoms and their treatment, either by contributing to the dopaminergic alterations directly or indirectly, or by creating a different pathway to pathology. For each system, the main findings in schizophrenia are reviewed, followed by a discussion of how such findings may affect dopamine transmission, at least to the extent that these interactions are known. Whenever possible, inferences to treatment are made, resulting in a review of potential new therapeutic targets.

One possible conclusion that emerges from this review of various contributions of many systems to schizophrenia pathology is that the dysregulation in DA may be a consequence of other upstream events. New research has shed light on interactions with glutamate and a deficient NMDA system leading to both DA alterations observed in schizophrenia, including cortical deficit and subcortical excess. These are reviewed by Daniel C. Javitt in his chapter. Similarly NMDA antagonists have been recently shown to engender some of the alterations in a subset of GABA neurons in the dorsolateral prefrontal cortex that have been described in schizophrenia (see the chapter by Lewis and Hashimoto), leading to a deficit in the $GABA_A$ α_2 subunit function and deficits in perisomatic inhibitory regulation of pyramidal neurons. Thus, these glutamatergic and GABAergic alterations are intimately linked and could lead to an inefficient control of cortical input onto subcortical striatal dopamine, as well as inefficient corticocortical connectivity and function. However, causality is difficult to assess in the presence of reciprocal regulations. Alterations in striatal dopamine transmission may itself affect cortical functioning by impairing glutamatergic flow of information in the corticostriatal-thalamocotical loops as illustrated recently by the genetically altered mice overexpressing striatal D2 leading to long-lasting cognitive deficits (Kellendonk *et al.*, 2006).

The role of 5-HT in schizophrenia is addressed by reviewing alterations in clinical studies: postmortem, pharmacological challenges and imaging studies (see the chapter by Abi-Dargham). As no clear patterns emerge for consistent findings, the emphasis seems to be better placed on the role that serotonin may play by modulating dopamine transmission in the critical brain regions implicated in schizophrenia and/or how it may affect response independently of its

role in modulating dopamine transmission. As reviewed by Marek in his chapter, DA–5-HT interactions in the brain are present at different anatomical levels, are mediated by different 5-HT receptor subtypes, and affect different aspects of DA function. This complexity leads to a rich potential pharmacology of cognitive enhancement with 5-HT_{1A} partial agonists, 5-HT_{2A} antagonists, 5-HT_4 partial agonists and 5-HT_6 antagonists.

While there have been many advances in the field of genetics and imaging contributing to our understanding of the basic pathophysiology of schizophrenia and its treatment, much remains to be discovered. Our therapeutic interventions are very limited in scope and in efficacy. The future seems to lie in all the unexplored potential targets that emerge from a systematic review of all systems, from D1 agonists, to $GABA_A$ α_2-specific allosteric modulators, to the many targets within the glutamate and serotonin systems. The modulation of the histaminergic system by antipsychotics and the antipsychotic-like properties of H_3-receptor antagonists/inverse agonists support a role of histamine neurons in schizophrenia (see the chapter by Arrang).

The following chapter reviews the evidence for a dysregulation of central cholinergic signaling in the pathophysiology of schizophrenia and suggests potential therapeutic roles for cholinergic targets (chapter by Berman, Talmage, and Role). This is followed by an in-depth review and analysis of one of these targets, the $\alpha 7$ nicotinic receptor (chapter by Martin and Freedman). The cannabinoid system and its relevance to schizophrenia is another emerging and exciting field, reviewed by D'Souza in his chapter. The connections between cannabinoids system and other neurotransmitters including dopamine are likely to be relevant as this system is present in all areas of the brain incriminated in schizophrenia, hippocampus, amygdala, prefrontal cortex, and striatum, where it exerts direct control over transmitter release. Endocannabinoids are released from lipid precursors in a receptor-dependent manner and serve as retrograde signaling messengers in GABAergic and glutamatergic synapses, as well as modulators of postsynaptic transmission, interacting with other neurotransmitters, including dopamine. A comprehensive review of clinical data linking alterations in neuropeptide systems to the etiology, pathophysiology, and treatment of schizophrenia in the chapter by Cáceda, Kinkead, and Nemeroff follows with a summary of potential therapeutic targets within this complex field. Genetic and postmortem evidence suggest a role for BDNF in the pathophysiology of schizophrenia. Interestingly, this neurotrophin controls the expression of the D3 receptor. The evidence supporting a role for BDNF in schizophrenia and the role of this neurotrophin at modulating dopamine transmission are reviewed in the chapter by Guillin, Demily, and Thibaut.

Finally, no review is complete without mention of the recent explosion of new candidate genes that may be affected in schizophrenia. Gogos in his chapter reviews the genetic contributions to schizophrenia and highlights the fact that

the neurobiology resulting from small variations in common genes is likely to be subtle and complex. This supports action at multiple molecular targets to reach an effective threshold in a large fraction of patients.

As many receptors modulate the same intracellular pathways, either synergistically or in an opposing manner, the final common pathway in schizophrenia may well be the signal transduction pathway(s) linked to these various neuroreceptor systems. Developing the appropriate tools to study intracellular targets in the living human brain is needed to understand how these alterations are integrated and lead to common symptomatology.

This book emphasizes what we know as well as all the many areas that need further research, that is, all the unknowns. In doing so, we hope it will provide a useful tool to the clinician and the researcher alike.

ANISSA ABI-DARGHAM
OLIVIER GUILLIN

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NEUROBIOLOGY OF DOPAMINE IN SCHIZOPHRENIA

Olivier Guillin,* Anissa Abi-Dargham,*,† and Marc Laruelle*,†

*Department of Psychiatry, Columbia College of Physicians and Surgeons
New York State Psychiatric Institute, Columbia University, New York 10032, USA
†Department of Radiology, Columbia College of Physicians and Surgeons
New York State Psychiatric Institute, Columbia University, New York 10032, USA

This chapter is an update on the dopamine (DA) imbalance in schizophrenia, including the evidence for subcortical hyperstimulation of D2 receptors underlying positive symptoms and cortical hypodopaminergia-mediating cognitive disturbances and negative symptoms. After a brief review of the anatomical neurocircuitry of this transmitter system as a background, we summarize the evidence for dopaminergic alterations deriving from pharmacological, postmortem, and imaging studies. This evidence supports a prominent role for D2 antagonism in the treatment of positive symptoms of schizophrenia and strongly suggests the need for alternative approaches to address the more challenging problem of negative symptoms and cognitive disturbances.

I. Introduction

Schizophrenia is a severe and chronic mental illness, associated with high prevalence (about 1% of the general population). Symptoms of schizophrenia typically emerge during adolescence or early adulthood. They are usually classified as positive, negative, or cognitive symptoms. Positive symptoms include: hallucinations, delusions, and severe thought disorganization. Negative symptoms

INTERNATIONAL REVIEW OF NEUROBIOLOGY, VOL. 78
DOI: 10.1016/S0074-7742(06)78001-1

0074-7742/07 $35.00

are a group of deficits comprising many dimensions such as affect (flattening), volition (apathy), speech (poverty), pleasure (anhedonia), and social life (withdrawal). Cognitive symptoms, such as deficits in attention and memory, are prominent features of the illness.

While the etiology and pathophysiology of schizophrenia remain unclear, a large body of evidence suggests that alterations in several neurotransmitter systems (e.g., dopamine, glutamatate, GABA, serotonin, cholinergic system, and others) are involved in the pathophysiological processes leading to the expression of these symptoms. Among these, the dopamine (DA) system has received most attention.

The involvement of DA in the pathophysiology and treatment of schizophrenia has been the subject of intense research efforts over the last 50 years. The first formulation of the DA hypothesis of schizophrenia proposed that hyperactivity of DA transmission was responsible for the core or "positive" symptoms (hallucinations, delusions) observed in this disorder (Carlsson and Lindqvist, 1963). This hypothesis was based on the correlation between clinical doses of antipsychotic drugs and their potency to block DA D2 receptors (Creese *et al.*, 1976; Seeman and Lee, 1975) and by the psychotogenic effects of DA-enhancing drugs (for review see Angrist and van Kammen, 1984; Lieberman *et al.*, 1987a). Given the predominant localization of DA terminals and D2 receptors in subcortical regions such as the striatum and the nucleus accumbens, the classical DA hypothesis of schizophrenia was concerned mostly with these subcortical regions.

Over the years, the increasing awareness of the importance of enduring negative and cognitive symptoms in this illness and of their resistance to D2 receptor antagonism has led to a reformulation of this classical DA hypothesis. Functional brain imaging studies suggested that these symptoms might arise from altered prefrontal cortex (PFC) functions (for reviews see Knable and Weinberger, 1997). A wealth of preclinical studies emerged documenting the importance of prefrontal DA transmission at D1 receptors (the main DA receptor in the neocortex) for optimal PFC performance (for review see Goldman-Rakic *et al.*, 2000). Together, these observations led to the hypothesis that a deficit in DA transmission at D1 receptors in the PFC might be implicated in the cognitive impairments and negative symptoms of schizophrenia (Davis *et al.*, 1991; Weinberger, 1987).

Thus, the current predominant view in that DA systems in schizophrenia might be characterized by an imbalance between subcortical and cortical DA systems: subcortical mesolimbic DA projections might be hyperactive (resulting in hyperstimulation of D2 receptors and positive symptoms) while mesocortical DA projections to the PFC might be hypoactive (resulting in hypostimulation of D1 receptors, negative symptoms, and cognitive impairment). Furthermore, since the seminal work of Pycock *et al.* (1980), many laboratories have described reciprocal and opposite regulations between cortical and subcortical DA systems (for review see Tzschentke, 2001). An abundant literature suggests that prefrontal DA activity exerts an inhibitory influence on subcortical DA activity (Deutch *et al.*, 1990;

Karreman and Moghaddam, 1996; Kolachana *et al.*, 1995; Wilkinson, 1997). From these observations, it has been proposed that, in schizophrenia, both arms of the DA imbalance model might be related, inasmuch as a deficiency in mesocortical DA function might translate into disinhibition of mesolimbic DA activity (Weinberger, 1987).

Despite decades of effort to generate experimental data supporting these hypotheses, documentation of abnormalities of DA function in schizophrenia has been difficult. Postmortem studies measuring DA and its metabolites and DA receptors in the brains of patients with schizophrenia yielded inconsistent or inconclusive results (for review see Davis *et al.*, 1991). Over the last few years, the development of new brain imaging methods based on the principle of endogenous competition enabled direct measurement of DA transmission at D2 receptor in the striatum (for review see Laruelle, 2000a). Combined with studies that documented increased striatal [^{18}F]dopa accumulation in schizophrenia, application of these new techniques to the study of schizophrenia provided new information into subcortical DA function dysregulation in schizophrenia (for review see Weinberger and Laruelle, 2001). Imaging studies have consistently demonstrated that schizophrenia is associated with increased presynaptic activity of DA neurons projecting to the striatum. Thus, the first arm of the dopaminergic imbalance hypothesis (hyperactivity in subcortical territory) has received strong support from imaging studies.

On the other hand, the second arm of this hypothesis (DA deficit in cortical projections) is still largely based on inferences from preclinical model or indirect clinical evidence. In contrast to the striatum, presynaptic DA function in the PFC is not at present accessible to noninvasive imaging techniques. D1 receptor availability is the only parameter of prefrontal DA function that is currently quantifiable *in vivo* with adequate reliability. Despite the limited information that this parameter provides to characterize DA function, PET imaging studies have described interesting relationships between alterations of D1 receptor availability and cognitive functions in schizophrenia (Abi-Dargham *et al.*, 2002; Karlsson *et al.*, 2002; Okubo *et al.*, 1997).

The goal of this chapter is to review current evidence for DA dysregulation in schizophrenia. Following a brief review of dopaminergic systems and receptors, pharmacological, postmortem, and imaging data that implicate DA alterations in schizophrenia will be presented.

II. Dopaminergic System in the Brain

A. Dopaminergic Projections

Dopaminergic projections are classically divided in nigrostriatal, mesolimbic, and mesocortical systems (Lindvall and Björklund, 1983). The nigrostriatal system projects from the substantia nigra (SN) to the dorsal striatum and has been

classically involved in cognitive integration, habituation, sensorimotor coordination, and initiation of movement. The mesolimbic system projects from the ventral tegmental area (VTA) to limbic structures such as ventral striatum, hippocampus, and amygdala. The mesocortical system projects from the VTA to cortical regions, mostly orbitofrontal, medial prefrontal, and cingulate cortices, but also to the dorsolateral prefrontal cortex (DLPFC), temporal, and parietal cortex. The mesolimbic and mesocortical systems are involved in regulation of motivation, attention, and reward (Mogenson *et al.*, 1980).

Corticostriatal–thalamocortical loops are important targets of DA modulation (Fig. 1). The general scheme of these loops involves projections from the cortex to striatum to the internal segment of the globus pallidum (GPi) or the SN pars

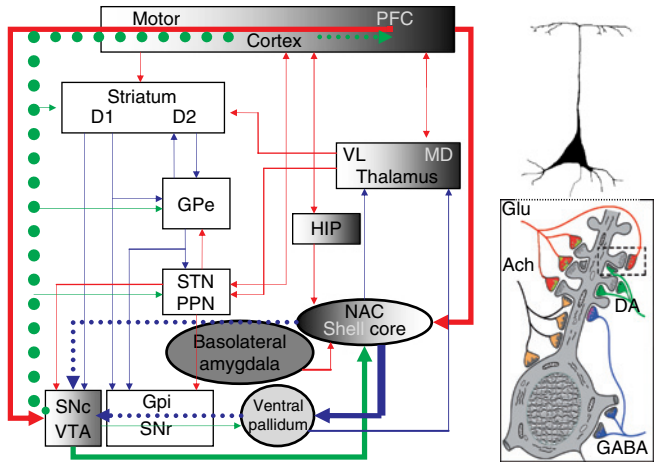

FIG. 1. Schematic representation of ventral limbic circuits implicated in the positive symptoms of schizophrenia. The primary neurotransmitter content and the major projections are represented by the colored arrows: blue, GABA; red, glutamate; green, DA. The nucleus accumbens (NAC) receives major excitatory inputs from prefontal cortex (PFC), basolateral amygdala, and hippocampus (HIP) and DA input from ventral tegmental area (VTA). NAC sends inputs to the ventral pallidum (VP) which sends, among others, inputs to the mediodorsal nucleus (MD) of the thalamus. MD provides excitatory inputs back to PFC. The NAC is a crucial node in this circuit where inputs from the PFC are gated by excitatory projections from HIP and BLA and DA projections from VTA. It is proposed that, in schizophrenia, increased DA activity induces perturbation in the information flow within this circuit, in the modulation of this information by hippocampus and amygdala, and in the gating of sensory information by the thalamus. If sustained, the increase in DA activity might lead to neuroplastic changes in this circuit resulting in the emergence of psychotic experience. Antipsychotics are antagonists at the D2 receptor which is expressed in the motor circuits represented by the loops involving the striatum (caudate and putamen), the external (GPe) and internal (GPi) globus pallidus, the substancia nigra pars compacta (SNc) and pars reticulata (SNr), and the pedoculopontine nucleus (PPN) and subthalamic nucleus (STN), explaining some of their motor side effects.

reticulata (SNr) to thalamus and back to the cortex. These loops have been classified into "limbic" loops (medial prefrontal and orbitofrontal cortex–ventral striatum–ventral pallidum–mediodorsal thalamic nuclei–cortex), associative loops (DLPFC–head of the caudate–GPi/SNr–ventral anterior thalamic nuclei–cortex), and motor loops (premotor and motor areas–putamen and body of the caudate–GPi/SNr–ventral anterior thalamic nuclei back–cortex) (Alexander *et al.*, 1986; Ferry *et al.*, 2000; Hoover and Strick, 1993; Joel and Weiner, 2000; Parent and Hazrati, 1995). The amygdala and hippocampus provide significant inputs to the ventral striatum, contributing to information integration into the limbic loop (Everitt *et al.*, 1991; Grace, 2000; Kunishio and Haber, 1994; Pennartz *et al.*, 1994). Animal studies suggest that the nucleus accumbens is the critical region in which both typical and atypical antipsychotic drugs exert their antipsychotic effects (Chiodo and Bunney, 1983; Deutch *et al.*, 1991; Robertson *et al.*, 1994). It is important to note that these different corticostriatal–thalamocortical loops are not completely segregated parallel loops. While corticostriatal–thalamic loops do generally reenter the cortical area that provides input to the striatal subregions involved in these loops, thus formed closed circuits and serving segregating processes, they also project back to other areas of the cortex, forming open circuits and serving integrative processes (Joel and Weiner, 2000).

Within each loop, the striatum output reaches the GPi/SNR via a direct pathway and via an indirect pathway that travels along the external segment of the globus pallidus (GPe) and the subthalamic nuclei (STN), both pathways providing antagonistic inputs to the GPi/SNr (Albin *et al.*, 1989; DeLong *et al.*, 1985; Gerfen, 1992; Joel and Weiner, 2000). The view of the antagonistic nature of the direct/stimulatory pathway versus the indirect/inhibitory pathway has been criticized as oversimplistic (Parent and Hazrati, 1995). Nevertheless, it is important to keep in mind that activation of medium spiny GABAergic neurons in the striatum by corticostriatal glutamatergic afferents can provide both stimulatory or inhibitory influences on thalamocortical projection (Carlsson *et al.*, 2001).

DA modulates the flow of information within these loops. In primates, DA cells from the VTA projects to the ventral striatum and cortex, the dorsal tier of the SN includes cells that project to all striatal regions and cortex, and the ventral tier of the SN projects widely throughout the dorsal striatum, but not to the cortex (for review see Haber and Fudge, 1997). The striatum provides GABA projections back to the VTA and SN. Projections from the VST to midbrain DA neurons are not restricted to the VTA and dorsal tier of the SN (where DA neurons projecting to the VST are located) but also terminate in the ventral tier of SN (where DA neurons projecting to the dorsal striatum are located). On the basis of these observations, Haber proposed that the DA system provides a bridge by which information circulating in the ventral limbic corticostriatal–thalamocortical loops spirals along nigrostriatal loops and feeds into the

cognitive and sensorimotor loops, translating drives into actions (Haber and Fudge, 1997; Haber *et al.*, 2000).

B. Dopaminergic Receptors

The advent of molecular biology techniques in the late 1980s enabled the cloning of these two receptors (Bunzow *et al.*, 1988; Dearry *et al.*, 1990; Monsma *et al.*, 1990; Zhou *et al.*, 1990), as well as three newer DA receptors, termed D3, D4, and D5 receptors (Sokoloff *et al.*, 1990; Sunahara *et al.*, 1991; Tiberi *et al.*, 1991; Van Tol *et al.*, 1991). On the basis of their sequence homologies, the five DA receptor subtypes were classified into two categories (Table I), a D1-like family (including D1 and D5 receptors), and a D2-like family (D2, D3, and D4 receptors) (for reviews see Civelli *et al.*, 1993; Gingrich and Caron, 1993; Sokoloff *et al.*, 1995). This classification is also coherent with the initial distinction of D1 and D2 receptors on the basis of their signaling system, that is, their coupling to Gs and Gi proteins and opposite effect on adenylyl cyclase (Kebabian and Calne, 1979; Spano *et al.*, 1978). D2-like family receptors are both postsynaptic and presynaptic autoreceptors (Diaz *et al.*, 2000; Missale *et al.*, 1998; Palermo-Neto, 1997).

DA receptors differ in their regional localization in the human brain (for reviews see Joyce and Meador-Woodruff, 1997; Meador-Woodruff *et al.*, 1996). D1 receptors show a widespread neocortical distribution, including the PFC, and are also present in high concentration in striatum. D5 receptors are concentrated in the hippocampus and entorhinal cortex (EC). D2 receptors are concentrated in the striatum, with low concentration in medial temporal structures (hippocampus, EC, amygdala) and thalamus. The concentration of D2 receptors in the PFC is extremely low. D3 receptors are present in the striatum, where their concentration is particularly high in the ventral striatum. D4 receptors are present in the PFC and hippocampus, but not detected in the striatum (Lahti *et al.*, 1998).

TABLE I
The D1-Like and D2-Like Family of DA Receptors

	D1-like		D2-like		
Receptor	D1	D5	D2	D3	D4
Sequence homology	60%		50–70%		
Gene organization	Intronless genes		Genes with intron		
Transduction	Stimulate adenylyl cyclase		Inhibit adenylyl cyclase		
Pharmacology	Moderate to low affinity for antipsychotics		High to moderate affinity for antipsychotics		

In the striatum, D2 receptors are preferentially found in enkephalin-rich GABAergic neurons that participate in the indirect pathways, while D1 receptors are most abundant in dynorphin/substance P GABAergic neurons that contribute to the direct pathways (Gerfen, 1992; Hersch *et al.*, 1995; Le Moine *et al.*, 1990, 1991). In rodents, D3 receptors are expressed in the Island of Calleja and in medium-sized spiny neurons of the rostral and ventromedial shell of nucleus accumbens (Diaz *et al.*, 1995), while its distribution in the striatum is more widespread in humans (Gurevich *et al.*, 1997). The magnitude of the segregation versus coexpression of D1 and D2 receptors in striatal neurons is still a matter of debate (Surmeier *et al.*, 1992, 1996). In the VST, D3 receptors colocalize preferentially on neurons expressing D1 receptors, substance P, dynorphin, and/or neurotensin (Diaz *et al.*, 1995; Ridray *et al.*, 1998) and TrkB, the high-affinity site for the brain-derived neurotrophic factor (BDNF) (Guillin *et al.*, 2001). In the shell of the accumbens, activation of D1 and D3 receptors results in a synergistic enhancement of substance P gene expression (Ridray *et al.*, 1998). In view of the high degree of coexpression of the two receptor subtypes in medium-sized spiny neurons of this region, it seems likely that the synergism occurs at the single-cell level and reflects the participation of the MAP kinase pathway of D3 receptor signaling synergistically increased by the cAMP pathway of the D1 receptor. The segregation of D2 and D1 receptors on different and antagonistic pathways might account for the fact that activation of these receptors is often synergistic at the behavioral level (e.g., stimulation of both D1 and D2 receptors stimulate locomotion), while their effect on intracellular signaling (starting with adenylate cyclase activity) are opposite in many regards. For example, stimulation of D1 and D2 receptors increases or decreases DARP32 phosphorylation, induces or blocks c-fos expression, promotes or inhibits *N*-methyl-D-aspartate (NMDA) receptor function, respectively (Dunah and Standaert, 2001; Konradi, 1998; Leveque *et al.*, 2000; Nguyen *et al.*, 1992; Nishi *et al.*, 1997). Thus, activation of D2 receptors by DA might provide an inhibitory influence to the indirect pathway and activation of D1 receptors by DA might provide a stimulatory influence on the direct pathway. Both effects are expected to result in stimulation of thalamocortical neurons.

However, the action of DA on target neurons should not be viewed in terms of simple excitation or inhibition. Unlike classical "fast" transmitters, DA does not directly gate ion channels, but stimulation of DA G-protein–linked receptor induces a cascade of intracellular signaling that results in modifying the response of the cells to other transmitters. DA is neither "inhibitory" or "excitatory," but its action will depend on the state of the neurons at the time of the stimulation (Yang *et al.*, 1999). Cortical glutamatergic afferents and DA projections converge on GABAergic medium spiny neurons in the striatum, usually on dendritic shafts and spines (for review see Kotter, 1994; Smith and Bolam, 1990; Starr, 1995). At this convergence point, DA has potent modulatory effects on glutamatate (Glu) transmission (for review see Cepeda and Levine, 1998; Konradi and

Heckers, 2003; Nicola *et al.*, 2000). Overall, D2 receptor stimulation inhibits NMDA-mediated Glu transmission and long-term potentiation (LTP), and D1 receptor stimulation facilitates Glu transmission and LTP (Centonze *et al.*, 2001; Levine *et al.*, 1996). The effect of D2 receptor stimulation on Glu transmission involves both pre- and postsynaptic effects: D2 stimulation inhibits Glu release and reduces the excitability of medium spiny neurons (Cepeda and Levine, 1998; Cepeda *et al.*, 2001; Leveque *et al.*, 2000; Nicola *et al.*, 2000; Onn *et al.*, 2000; Peris *et al.*, 1988; West and Grace, 2002). In contrast, D1 receptor stimulation generally promotes NMDA function and medium spiny neuron excitability, more specifically when the cells are in a depolarized "upstate," due to the convergence of excitatory inputs (Dunah and Standaert, 2001; Flores-Hernandez *et al.*, 2002; Hernandez-Lopez *et al.*, 1997; Marti *et al.*, 2002; Morari *et al.*, 1994; West and Grace, 2002; Wilson and Kawaguchi, 1996; Fig. 2).

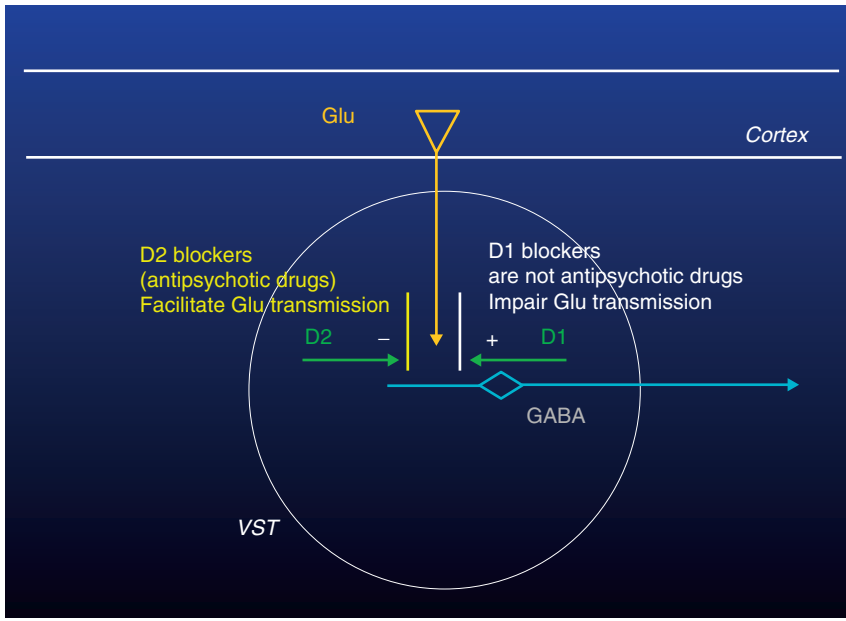

FIG. 2. Opposite modulations of NMDA transmission by D2 and D1 receptors in GABAergic medium spiny neurons in the striatum. D2 and D1 receptors inhibit and facilitate, respectively, Glu transmission. Thus, an excess of D2 receptor stimulation in schizophrenia would further impair NMDA-mediated information flow from the cortex into the striatum. By blocking D2 receptors, antipsychotic drugs promote NMDA transmission. Conversely, D1 receptor antagonists weaken NMDA transmission and are not antipsychotic drugs.

In the PFC, $D_{1/5}$ receptors are localized both on pyramidal cells (dendritic spines and shafts) and on axonal terminals of nondopaminergic neurons (Smiley *et al.*, 1994), while some data suggest that D4 receptors might be localized on GABA interneurons (Mrzljak *et al.*, 1996). DA modulates pyramidal cell excitability, both directly and via GABAergic interneurons (Yang *et al.*, 1999). Recent data suggest that DA differently affect GABAergic activity in the PFC via D1- or D2-like mechanisms, whereas $D_{1/5}$ and $D_{2/4}$ receptor stimulation enhances or inhibits GABAergic activity, respectively. Here again, it has been proposed that DA increases the signal-to-noise ratio of glutamatergic afferents (Seamans *et al.*, 2001).

III. Evidence Supporting Alterations of DA Systems in Schizophrenia

A. Pharmacological Evidence

1. *Aversive Pharmacological Effects*

The psychotogenic effect of amphetamine and other DA-enhancing drugs, such as methylphenidate and L-dopa, is a cornerstone of the classical DA hypothesis of schizophrenia. Two sets of observations are relevant to this issue. First, repeated exposure to high doses of psychostimulants in nonschizophrenic subjects might gradually induce paranoid psychosis. This well-documented observation shows that sustained increase in DA activity is psychotogenic. Second, low doses of psychostimulants that are not psychotogenic in healthy subjects might induce or worsen psychotic symptoms in patients with schizophrenia. This observation indicates that patients with schizophrenia have an increased vulnerability to the psychotogenic effects of DA-enhancing drugs.

a. Amphetamine-Induced Psychosis in Nonschizophrenic Subjects. Although mentioned in 1938 (Young and Scoville, 1938), amphetamine-induced psychosis was not clearly recognized as a possible consequence of chronic amphetamine use until 1958 on the publication of a 42-case monograph by Connell (1958). In this chapter, Connell provided the "classical" definition of amphetamine psychosis, as "*a paranoid psychosis with ideas of references, delusions of persecution, auditory and visual hallucinations in the setting of a clear sensorium*" and concluded that "*the mental picture may be indistinguishable from acute or chronic paranoid schizophrenia*" (Connell, 1958).

In the early 1970s, several studies experimentally induced amphetamine psychosis in nonschizophrenic amphetamine-abusers in order to better document the clinical pattern of this syndrome (Angrist and Gershon, 1970; Bell, 1973; Griffith *et al.*, 1968). These experiments formally established that sustained psychostimulant exposure can produce paranoid psychosis in nonschizophrenic

individuals. This reaction does not occur in the context of a delirium since subjects maintain a clear sensorium during the episode and are able to recollect the episode after its resolution. Since these studies were performed before the conceptualization of the symptoms of schizophrenia into positive and negative (Crow, 1980), they did not formally assess negative symptoms. These papers only include anecdotal reports of emotional blunting, withdrawal, or alogia, thereby, suggesting that sustained and excessive stimulation of DA systems does not consistently induce what is now defined as the "negative" symptoms of schizophrenia.

Ellinwood (Ellinwood 1967; Ellinwood *et al.*, 1973) provided one of the most insightful descriptions of amphetamine-induced psychosis by conceptualizing the condition as a continuum that evolves from the gradual onset of paranoid tendencies to delusional paranoia. The first step is characterized by stimulation of interpretative mental activities (great attention to details, intense feeling of curiosity, repetitive searching, and sorting behavior). Ellinwood sees in Sherlock Holmes, a regular cocaine user, a prototypical example of the endless search for meanings (*my mind rebels at stagnation*). With increased exposure, these paranoid tendencies and interests for the minutiae develop into an intermediate stage, which is characterized by marked enhancement of perceptual acuity, sustained "pleasurable" suspiciousness, and compulsive probing behavior. Finally, this inquisitive behavior is reversed and projected to others (persecution), leading to paranoia and ideas of references. The "enhancement of sensitive acuity" develops into hallucinations, initially auditory, then visual and tactile. The sensorium remains clear until toxic delirium is reached. Thought disorders might manifest toward the end of the continuum near the toxic stage. Kapur (2003) recently reformulated and modernized the Ellinwood "Sherlock Holmes" theory by defining schizophrenia psychosis as a state of aberrant salience.

Another important property of psychostimulants is their ability to induce reverse tolerance or "sensitization" (Kalivas *et al.*, 1993; Robinson and Becker, 1986). Long-term sensitization to psychostimulants is a process whereby repeated exposure to these drugs results in an enhanced response on subsequent exposures. The relevance of this process for the pathophysiology of schizophrenia has been reviewed (Laruelle, 2000b; Lieberman *et al.*, 1997). Subjects who abused psychostimulants and experienced stimulant-induced psychotic episodes are reported to remain vulnerable to low doses of psychostimulants (Connell, 1958; Ellinwood *et al.*, 1973; Sato *et al.*, 1983). In these subjects, exposure to psychostimulants at doses that do not normally produce psychotic symptoms can trigger a recurrence of these symptoms. The similarity between these patients and the patients with schizophrenia in terms of vulnerability to the psychotogenic effects of psychostimulants has led to the theorization that schizophrenia might be associated with an "endogenous" sensitization process (Glenthoj and Hemmingsen, 1997; Laruelle, 2000b; Lieberman *et al.*, 1990).

Considerable research efforts have been devoted to the identification of neuronal substrates involved in sensitization. Several studies have shown that sensitization is associated with increased stimulant-induced DA release in the axonal terminal fields (for references see Laruelle, 2000b). A brain imaging study confirmed that, in humans, sensitization to the effects of amphetamine involves increased amphetamine-induced DA release (Boileau *et al.*, 2003). The imaging studies reviewed below show that patients with schizophrenia display an enhanced amphetamine-induced DA release, supporting the notion of an endogenous sensitization process of subcortical DA system in schizophrenia.

b. Psychotogenic Effects of Amphetamine in Schizophrenic Patients. A number of studies reviewed by Lieberman *et al.* (1987b) provided evidence that patients with schizophrenia, as a group, display increased sensitivity to the psychotogenic effects of acute psychostimulant administration. In other terms, some, but not all patients with schizophrenia present emergence or worsening of psychotic symptoms after acute exposure to psychostimulants at doses that do not induced psychosis in healthy subjects. The psychotic response appears to be state dependent. First, patients who responded with a psychotic reaction to a psychostimulant challenge during an acute episode failed to show such a response when they were in remission. Second, the propensity to present a psychotic reaction to a psychostimulant challenge is predictive of relapse on antipsychotic discontinuation. Thus, the clinical response to stimulants might "reveal" an active phase of the illness that is not readily identifiable by the clinical symptomatology in the absence of a psychostimulant administration.

2. *Therapeutic Pharmacological Effects*

Since the recognition in 1952 of the antipsychotic properties of chlorpromazine (Delay *et al.*, 1952), antipsychotic medications have fundamentally altered the course and the prognosis of schizophrenia. They have proven effective at reducing the severity of symptoms and preventing episodes of illness exacerbation. To date, D2 receptor antagonism is the only pharmacological property shared by all antipsychotic drugs. The clinical dose of these drugs is related to their affinity for D2 receptors. D2 receptor antagonism appears both necessary and sufficient for antipsychotic action (as demonstrated by the selective D2 receptor antagonist amisulpride). The fact that patients with schizophrenia improve following administration of D2 receptor antagonists is one of the few irrefutable pieces of evidence in schizophrenia (Weinberger, 1987).

D2 receptor blockade by antipsychotic drugs has been confirmed by a large number of imaging studies (reviewed in Talbot and Laruelle, 2002). In general, these studies failed to observe a relationship between the degree of D2 receptor occupancy and the quality of the clinical response. However, most studies reported doses achieving more than 50% occupancy. The minimum occupancy required for a therapeutic response remains somewhat uncertain. Two studies

performed with low doses of relatively selective D2 receptor antagonists (haloperidol and raclopride) suggest that a minimum of 50% occupancy is required to observe a rapid clinical response (Kapur *et al.*, 2000; Nordstrom *et al.*, 1993). Imaging studies have repeatedly confirmed the existence of a striatal D2 receptor occupancy threshold (about 80%) above which extrapyramidal symptoms (EPS) are likely to occur (Farde *et al.*, 1992). Thus, these data suggest the existence of a therapeutic window between 50% and 80% striatal D2 receptor occupancy. Within this window, the relationship between occupancy and response is unclear, presumably because the variability in endogenous DA (Frankle *et al.*, 2004). Furthermore, the occupancy threshold required for therapeutic effects might differ among drugs.

The introduction of a second generation of antipsychotic (SGA) drugs since the early 1990s has not fundamentally altered the prominence of D2 receptor antagonism in the current treatment of schizophrenia. Most SGAs also potently interact with other receptors, such as the serotonin 5-HT_{2A} receptors, but the possibility to achieve an "atypical" profile with a pure D2 receptor antagonist, such as amisulpride, indicates that serotonin pharmacological effects are not absolutely required to produce this effect.

On the other hand, imaging studies have generally reported lower occupancies of striatal D2 receptors at therapeutic doses of SGAs compared to first generation antipsychotic drugs (FGAs). This seems to be especially true for amisulpride, clozapine, and quetiapine, which provide 50–60% D2 receptor occupancy range at clinically effective doses (for review and references see Abi-Dargham and Laruelle, 2005). In contrast, studies with FGAs often reported occupancies exceeding 75%. Thus, a parsimonious hypothesis to account for the SGA superiority is that, in general, clinical results obtained after moderate occupancies (50–75%) are better than after high occupancies (75–100%), and that, for a variety of reasons, SGAs tend to maintain lower occupancies than FGAs. The alternate hypothesis is that the D2 receptor occupancy required for therapeutic effects is lower in SGAs than FGAs. Should the alternate hypothesis be true, the mechanisms responsible for the gain in the occupancy–efficacy relationship of SGAs remain to be fully elucidated.

A potentially important synergistic effect of 5-HT_{2A} and D2 receptor antagonism is to increase prefrontal DA, an effect not observed with selective D2 or 5-HT_{2A} receptor antagonists administered alone (Gessa *et al.*, 2000; Ichikawa *et al.*, 2001; Melis *et al.*, 1999; Pehek and Yamamoto, 1994; Youngren *et al.*, 1999). This effect might be mediated by the stimulation of 5-HT_{1A} receptors: it is blocked by 5-HT_{1A} antagonists and is also observed following the combination of 5-HT_{1A} receptor agonism and D2 receptor antagonism (Ichikawa *et al.*, 2001; Rollema *et al.*, 2000). Aripiprazole, clozapine, quetiapine, and ziprasidone are also 5-HT_{1A} partial agonists, and this additional property might also contribute to their ability to increase prefrontal DA. As discussed in Section III.B, a decreased prefrontal

DA function might contribute to the cognitive deficits present in patients with schizophrenia, and it is possible that an increase in prefrontal DA induced by SGAs might mediate some of the modest cognitive improvements induced by these drugs (Keefe *et al.*, 1999). Yet, it is unclear whether this increase in prefrontal DA, documented as an acute response in animal studies, is sustained during the course of treatment in patients with schizophrenia.

B. Postmortem Studies

The discovery of the antipsychotic effect of D2 receptor blockade inspired numerous postmortem studies seeking to determine whether schizophrenia was associated with altered parameters of DA transmission. These postmortem studies have for the most part failed to provide definitive answers, partly because of the confounding effects of antemortem antipsychotic treatment.

1. *Tissue DA and HVA*

Direct measures of tissue content of DA and its metabolites have failed to demonstrate consistent and reproducible abnormalities (for review see Davis *et al.*, 1991; Reynolds, 1989). It should be noted, however, that some studies have reported higher DA tissue levels in samples from patients with schizophrenia in subcortical regions such as caudate (Owen *et al.*, 1978), nucleus accumbens (Mackay *et al.*, 1982), or amygdala (Reynolds, 1983).

2. *D2 Receptors*

Increased density of striatal D2 receptors in patients with schizophrenia has been a consistent finding in a large number of postmortem studies (Cross *et al.*, 1983; Dean *et al.*, 1997; Hess *et al.*, 1987; Joyce *et al.*, 1988; Knable *et al.*, 1994; Lahti *et al.*, 1996; Lee *et al.*, 1978; Mackay *et al.*, 1982; Marzella *et al.*, 1997; Mita *et al.*, 1986; Owen *et al.*, 1978; Reynolds *et al.*, 1987; Ruiz *et al.*, 1992; Seeman *et al.*, 1984, 1987, 1993; Sumiyoshi *et al.*, 1995). Because chronic neuroleptic administration upregulates D2 receptor density (Burt *et al.*, 1977), it is likely that these postmortem findings are related to prior neuroleptic exposure rather than to the disease process per se. In light of these very consistent results with [^{3}H]spiperone, it is interesting to note that the striatal binding of [^{3}H]raclopride has been reported to increase in many studies (Dean *et al.*, 1997; Marzella *et al.*, 1997; Ruiz *et al.*, 1992; Sumiyoshi *et al.*, 1995), but normal in several others (Knable *et al.*, 1994; Lahti *et al.*, 1996; Seeman *et al.*, 1993), even in patients exposed to neuroleptic drugs prior to death. This observation suggests that the increase in [^{3}H]raclopride binding is of lower magnitude than the one of [^{3}H]spiperone binding. This discrepancy might simply reflect the observation that, for reasons that are not currently understood, antipsychotic drugs upregulate more [^{3}H]spiperone

than [^{3}H]raclopride binding to D2 receptors (Schoots *et al.*, 1995; Tarazi *et al.*, 1997).

3. *D3 Receptors*

A significant increase in D3 receptor number in VST samples from patients with schizophrenia who were off neuroleptics at the time of death has been reported in one study (Gurevich *et al.*, 1997). In contrast, in patients who had been treated with neuroleptics up to the time of death, D3 receptor levels did not differ significantly from those of controls (Gurevich *et al.*, 1997). These data were interpreted as indicating that antipsychotics downregulate the D3 receptor in schizophrenic patients who otherwise have a higher density of this receptor in the VST. The D3 receptor gene expression is under the control of a neutrophin, called BDNF, that is synthesized either in the VTA and the PFC and released in the VST, where it maintains the expression of the D3 receptor (Guillin *et al.*, 2001). One study (Takahashi *et al.*, 2000) has shown increased and two decreased (Hashimoto *et al.*, 2005; Weickert *et al.*, 2003) of BDNF levels in the brain of patients with schizophrenia. D3 receptors are upregulated in the presence of hyperdopaminergic tone (Bordet *et al.*, 1997; Fauchey *et al.*, 2000; Guillin *et al.*, 2001; Le Foll *et al.*, 2002), under the control of the BDNF, whose synthesis is in turn under the control of the activity of neurons projecting from the PFC or the VTA in the VST.

4. *D4 Receptors*

On the basis of ligand subtraction techniques, several studies have reported increased D4-like receptors in schizophrenia (Marzella *et al.*, 1997; Murray *et al.*, 1995; Seeman *et al.*, 1993; Sumiyoshi *et al.*, 1995). These findings were not confirmed by other studies using the same technique (Lahti *et al.*, 1996; Reynolds and Mason, 1994), nor by a study using [^{3}H]NGD 94-1, a selective D4 ligand (Lahti *et al.*, 1998). Moreover, the hypothesis that clozapine might act by blocking the D4 receptor was not supported by a clinical trial with the D4 selective agent L745,870 (Kramer *et al.*, 1997).

5. *D1 Receptors*

Striatal D1 receptors have generally been reported to be unaltered in schizophrenia (Joyce *et al.*, 1988; Pimoule *et al.*, 1985; Reynolds and Czudek, 1988; Seeman *et al.*, 1987), although one study reported decreased density (Hess *et al.*, 1987). In the PFC, one study reported no changes (Laruelle *et al.*, 1990) and one reported a nonsignificant increase (Knable *et al.*, 1996).

6. *DA Transporter*

A large number of studies have reported unaltered DA transporter density (DAT) in the striatum of patients with schizophrenia (Chinaglia *et al.*, 1992;

Czudek and Reynolds, 1989; Hirai *et al.*, 1988; Joyce *et al.*, 1988; Knable *et al.*, 1994; Pearce *et al.*, 1990).

7. *Tyrosine Hydroxylase Immunolabeling*

A recent and interesting postmortem finding regarding DA parameters in patients with schizophrenia is the observation of decreased tyrosine hydroxylase (TH)-labeled axons in layers III and VI of the EC and in layer VI of the PFC, a finding suggesting that schizophrenia might be associated with deficit in DA transmission in the EC and PFC (Akil *et al.*, 1999, 2000). This finding was clearly unrelated to premortem neuroleptic exposure. Benes *et al.* (1997) observed no significant changes in TH-positive varicosities in the DLPFC. In the anterior cingulate region (layer II), these authors observed a significant shift in the distribution of TH varicosities from large neurons to small neurons.

In conclusion, postmortem measurements of indices of DA transmission generated a number of consistent observations in the striatum: (1) The binding of radioligand to D2-like receptors in the striatum of patients with schizophrenia is increased, but the magnitude of this increase varies with the type of radioligands used, and it is difficult to exclude the contribution of premortem antipsychotic exposure in this set of findings. (2) Striatal DAT and D1 receptors density is unaffected in schizophrenia. Several interesting observations such as increase in D3 receptors in the ventral striatum and alteration in TH immunolabeling in several cortical regions do not appear to be consequences of premortem neuroleptic exposure, but these findings have yet to be independently confirmed.

C. Imaging Studies

1. *Striatal DA Function*

The development of PET and SPECT imaging techniques in the late 1980s made possible, for the first time, the examination of DA function *in vivo* in patients with schizophrenia never exposed to antipsychotic drugs (Fig. 3).

a. Striatal D2 and D1 Receptors. Striatal D2 receptor density in schizophrenia has been extensively studied with PET and SPECT imaging. In a meta-analysis (Weinberger and Laruelle, 2001), 17 imaging studies comparing D2 receptor parameters in patients with schizophrenia have been analyzed (included a total of 245 patients and 231 control subjects, Table II) (Abi-Dargham *et al.*, 1998, 2000b; Blin *et al.*, 1989; Breier *et al.*, 1997; Crawley *et al.*, 1986; Hietala *et al.*, 1994b; Knable *et al.*, 1997; Laruelle *et al.*, 1996; Martinot *et al.*, 1990, 1991; Pilowsky *et al.*, 1994; Wong *et al.*, 1986). Updated with a study (Yang *et al.*, 2004), this meta-analysis revealed a small (12%) but significant elevation of striatal D2 receptors in untreated patients with schizophrenia. No clinical correlates of increased D2 receptor-binding parameters could be identified. Studies performed with

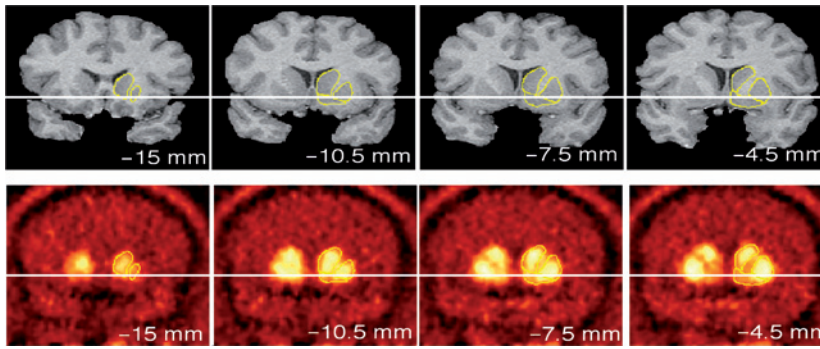

FIG. 3. D2 receptor-binding image with PET and [^{11}C]raclopride. MRI images (above) and PET images (below).

butyrophenones ($n = 7$) show an effect size of 0.96 ± 1.05, significantly larger than the effect size observed with other ligands (benzamides and lisuride, $n = 11$, 0.19 ± 0.25, $p = 0.02$). This difference might be due to differences in vulnerability of the binding of these tracers to endogenous DA and elevation of endogenous DA in schizophrenia (Seeman, 1988; Seeman *et al.*, 1989). Interestingly, the fact that D2 receptor levels are increased in healthy monozygotic twin compared to dizygotic twin of patients with schizophrenia has lead to the conclusion that the caudate DA D2 receptor upregulation is related to genetic risk for schizophrenia (Hirvonen *et al.*, 2005). Imaging studies of D1 receptors have consistently failed to detect abnormalities of D1 receptor availability in the striatum of patients with schizophrenia (Abi-Dargham *et al.*, 2002; Karlsson *et al.*, 2002; Okubo *et al.*, 1997).

b. Striatal Amphetamine-Induced DA Release. The decrease in [^{11}C]raclopride and [^{123}I]IBZM *in vivo* binding following acute amphetamine challenge has been well validated as a measure of the change in D2 receptor stimulation by DA due to amphetamine-induced DA release (Breier *et al.*, 1997; Laruelle *et al.*, 1997b; Villemagne *et al.*, 1999) (Table III).

Our results (Abi-Dargham *et al.*, 1998; Laruelle *et al.*, 1996), which have been independently replicated (Breier *et al.*, 1997), showed that the amphetamine-induced decrease in [^{11}C]raclopride or [^{123}I]IBZM binding is elevated in untreated patients with schizophrenia compared to well-matched controls (Fig. 4). A significant relationship was observed between the magnitude of this effect and transient induction or worsening of positive symptoms. This exaggerated response of the DA system to amphetamine was observed in both first episode/drug-naive patients and previously treated patients (Laruelle *et al.*, 1999), but was larger in

TABLE II
IMAGING STUDIES OF STRIATAL D2 RECEPTOR PARAMETERS IN DRUG-NAIVE AND DRUG-FREE PATIENTS WITH SCHIZOPHRENIA

Class radiotracer	Radiotracer	Study	Controls, *n*	Patients, *n* (DN/DF)[a]	Method	Outcome	Controls (*n*.mean ± SD)[b]	Patients (*n*.mean ± SD)[b]	*p*	Effect size[c]	Ratio SD
Butyrophenones	[^{11}C]NMSP	Wong *et al.* (1986)	11	15 (10/5)	Kinetic	*B*max	100 ± 50	253 ± 105	<0.05	3.06	2.10
	[^{76}Br]SPI	Crawley *et al.* (1986)	8	16 (12/4)	Ratio	S/C	100 ± 14	111 ± 12	<0.05	0.79	0.86
	[^{76}Br]SPI	Blin *et al.* (1989)	8	8 (0/8)	Ratio	S/C	100 ± 14	104 ± 14	ns	0.28	1.00
	[^{76}Br]SPI	Martinot *et al.* (1990)	12	12 (0/12)	Ratio	S/C	100 ± 11	101 ± 15	ns	0.14	1.41
	[^{11}C]NMSP	Tune *et al.* (1993)	17	10 (8/2)	Kinetic	*B*max	100 ± 80	173 ± 143	0.08	0.91	1.79
	[^{11}C]NMSP	Nordstrom *et al.* (1995)	7	7 (7/0)	Kinetic	*B*max	100 ± 25	133 ± 63	ns	1.33	2.50
	[^{11}C]NMSP	Okubo *et al.* (1997)	18	17(10/7)	Kinetic	k3	100 ± 21	104 ± 16	ns	0.19	0.74
Benzamides	[^{11}C]raclopride	Farde *et al.* (1990)	20	18 (18/0)	Equilibrium	*B*max	100 ± 29	107 ± 18	ns	0.23	0.63
	[^{11}C]raclopride	Hietala *et al.* (1994a)	10	13 (0/13)	Equilibrium	*B*max	100 ± 22	112 ± 43	ns	0.55	1.99
	[^{123}I]IBZM	Pilowsky *et al.* (1994)	20	20 (17/3)	Ratio	S/FC	100 ± 8	99 ± 7	ns	−0.07	0.82

(*Continued*)

TABLE II (*Continued*)

Class radiotracer	Radiotracer	Study	Controls, *n*	Patients, *n* (DN/DF)[a]	Method	Outcome	Controls (*n*.mean ± SD)[b]	Patients (*n*.mean ± SD)[b]	*p*	Effect size[c]	Ratio SD
	[^{123}I]IBZM	Laruelle *et al.* (1996)	15	15 (1/14)	Equilibrium	BP	100 ± 26	115 ± 33	ns	0.56	1.25
	[^{123}I]IBZM	Knable *et al.* (1997)	16	21 (1/20)	Equilibrium	BP	100 ± 29	97 ± 38	ns	−0.12	1.31
	[^{11}C]raclopride	Breier *et al.* (1997)	12	11 (6/5)	Equilibrium	BP	100 ± 18	100 ± 30	ns	0.02	1.69
	[^{123}I]IBZM	Abi-Dargham *et al.* (1998)	15	15 (2/13)	Equilibrium	BP	100 ± 20	102 ± 49	ns	0.09	2.50
	[^{123}I]IBZM	Abi-Dargham *et al.* (2000b)	18	18 (8/10)	Equilibrium	BP	100 ± 13	104 ± 14	ns	0.33	1.11
	[^{123}I]IBZM	Yang *et al.* (2004)	12	11(11/0)	Ratio	S/C	100 ± 11	101 ± 11	ns	0.09	1
Ergot alk.	[^{76}Br]lisuride	Martinot *et al.* (1991)	14	19 (10/9)	Ratio	S/C	100 ± 10	104 ± 12	ns	0.45	1.21
	[^{76}Br]lisuride	Martinot *et al.* (1994)	10	10 (2/8)	Ratio	S/C	100 ± 10	100 ± 13	ns	0.00	1.29

[a]DN, drug naive; DF, drug free.
[b]Mean normalized to mean of control subjects.
[c]Effect size calculated as (mean patients − mean controls)/SD controls.

TABLE III

IMAGING STUDIES OF STRIATAL PRESYNAPTIC DA PARAMETERS IN DRUG-NAIVE AND DRUG-FREE PATIENTS WITH SCHIZOPHRENIA

Parameter	Study	Controls, *n*	Patients, *n* (DN/DF/T)[a]	Radiotracer (per challenge)	Method	Outcome	Controls (*n*, mean ± SD)[b]	Patients (*n*, mean ± SD)[b]	*p*	Effect size[c]
DOPA accumulation	Reith *et al.* (1994)	13	5 (4/0/1)	[^{18}F]DOPA	Kinetic	k3	100 ± 23	120 ± 15	<0.05	0.91
	Hietala *et al.* (1995)	7	7 (7/0/0)	[^{18}F]DOPA	Graphical	Ki	100 ± 11	117 ± 20	<0.05	1.54
	Dao-Castellana *et al.* (1997)	7	6 (2/4/0)	[^{18}F]DOPA	Graphical	Ki	100 ± 11	103 ± 40	ns	0.30
	Lindstrom *et al.* (1999)	10	12 (10/2)	[^{11}C]DOPA	Graphical	Ki	100 ± 17	113 ± 12	<0.05	0.77
	Hietala *et al.* (1999)	13	10 (10/0)	[^{18}F]DOPA	Graphical	Ki	100 ± 14	115 ± 28	<0.05	1.09
	Elkashef *et al.* (2000)	13	19 (0/9/10)	[^{18}F]DOPA	Ratio	Ki	100 ± 11.7	92.4 ± 9.7	<0.05	−0.65
	Meyer-Lindenberg *et al.* (2002)	6	6 (0/6/0)	[^{18}F]DOPA	Graphical	Ki	100 ± 9.7	119 ± 9.7	<0.02	1.96
	McGowan *et al.* (2004)	12	16 (0/0/16)	[^{18}F]DOPA	Graphical	Ki	100 ± 9.3	115 ± 8.2	0.001	1.6
Amphetamine-induced DA release	Laruelle *et al.* (1996)	15	15 (2/13/0)	[^{123}I]IBZM/ amphetamine	Equilibrium	Delta BP	100 ± 113	271 ± 221	<0.05	1.51
	Breier *et al.* (1997)	18	18 (8/10/0)	[^{11}C]raclopride/ amphetamine	Equilibrium	Delta BP	100 ± 43	175 ± 82	<0.05	1.73
	Abi-Dargham *et al.* (1998)	16	21 (1/20/0)	[^{123}I]IBZM/ amphetamine	Equilibrium	Delta BP	100 ± 88	194 ± 145	<0.05	1.07

(*Continued*)

TABLE III (*Continued*)

Parameter	Study	Controls, *n*	Patients, *n* (DN/DF/T)[a]	Radiotracer (per challenge)	Method	Outcome	Controls (*n*, mean ± SD)[b]	Patients (*n*, mean ± SD)[b]	*p*	Effect size[c]
Baseline DA concentration	Abi-Dargham *et al.* (2000b)	18	18 (8/10/0)	[^{123}I]IBZM/ αMPT	Equilibrium	Delta BP	100 ± 78	211 ± 122	<0.05	1.43
DAT density	Laakso *et al.* (2000)	9	9 (9/0/0)	[^{18}F]CFT	Ratio	S/C	100 ± 12	101 ± 13	<0.05	0.11
	Laruelle *et al.* (2000a)	22	22 (2/20/0)	[^{123}I]CIT	Equilibrium	BP	100 ± 17	93 ± 20	<0.05	−0.43
	Hsiao *et al.* (2003)	12	12 (12/0/0)	[^{99}mTc]TRODAT	Ratio	S/Occ	100 ± 18	104 ± 21	ns	0.22

[a]DN, drug naive; DF, drug free; T, treated with antipsychotics.
[b]Mean normalized to mean of control subjects.
[c]Effect size calculated as (mean patients − mean controls)/SD controls.

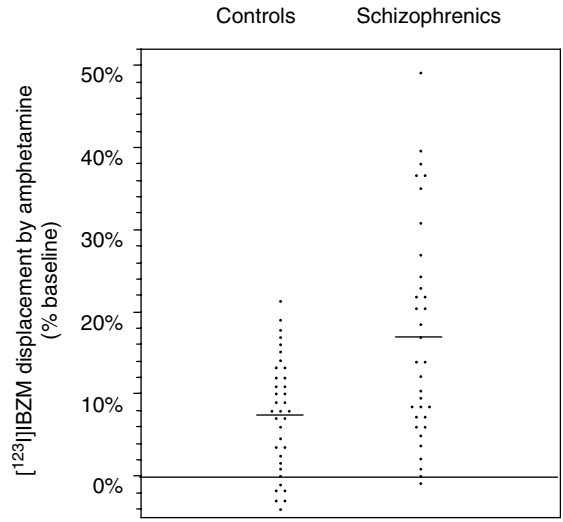

FIG. 4. Effect of amphetamine (0.3 mg/kg) on [^{123}I]IBZM binding in healthy controls and untreated patients with schizophrenia. The *y* axis shows the percentage decrease in [^{123}I]IBZM-binding potential induced by amphetamine, which is a measure of the increased occupancy of D2 receptors by DA following the challenge. Increased stimulation of D2 receptors in schizophrenia was associated with transient worsening or emergence of positive symptoms.

patients experiencing an episode of illness exacerbation than in patients in remission at the time of the scan (Laruelle *et al.*, 1999). This exaggerated DA reactivity did not appear to be a nonspecific effect of stress, as higher self-reports of anxiety before the experiments were not associated with larger effect of amphetamine on [^{123}I]IBZM binding. Furthermore, nonpsychotic subjects with unipolar depression, who reported levels of anxiety similar to the schizophrenic patients at the time of the scan, showed normal amphetamine-induced displacement of [^{123}I] IBZM (Parsey *et al.*, 2001).

These findings have generally been interpreted as reflecting an increase in synaptic DA following amphetamine in the schizophrenic group. Another interpretation of these observations would be that schizophrenia is associated with increased affinity of D2 receptors for DA.

c. DAT Transporters. Three imaging studies (listed in Table II) have confirmed the *in vitro* observation of normal striatal DAT density in schizophrenia (Laakso *et al.*, 2000; Laruelle *et al.*, 2000b). In addition, no association between amphetamine-induced DA release and DAT density was found (Laruelle *et al.*, 2000b), suggesting that the increased presynaptic output revealed by the studies reviewed above is not due to higher terminal density.

d. Vesicular Monoamine Transporter. Using the radiotracer [^{11}C]DTBZ (Taylor *et al.*, 2000) were not able to show any difference in vesicular monoamine transporter binding potential (BP) in patients with schizophrenia compared to healthy subjects.

e. Baseline Occupancy of Striatal D2 Receptors by DA. In rodents, acute depletion of synaptic DA is associated with an acute increase in the *in vivo* binding of [^{11}C]raclopride or [^{123}I]IBZM to D2 receptors (for review see Laruelle, 2000a). The increased binding is observed *in vivo* but not *in vitro*, indicating that it is not due to receptor upregulation (Laruelle *et al.*, 1997a) but to removal of endogenous DA and unmasking of D2 receptors previously occupied by DA. A similar acute DA depletion technique paired with D2 receptor imaging in humans using αHPT has been developed to assess the degree of occupancy of D2 receptors by DA (Laruelle *et al.*, 1997a). In schizophrenia, there was a higher occupancy of D2 receptors by DA in patients experiencing an episode of illness exacerbation, compared to healthy controls (Table III) (Abi-Dargham *et al.*, 2000b). Again assuming normal affinity of D2 receptors for DA, the data are consistent with higher DA synaptic levels in patients with schizophrenia. Higher synaptic DA levels in patients with schizophrenia were predictive of good therapeutic response of these symptoms following 6 weeks of treatment with atypical antipsychotic medications (Abi-Dargham *et al.*, 2000b).

f. Striatal DOPA Decarboxylase Activity. The eight studies which have reported rates of DOPA decarboxylase in patients with schizophrenia, using [^{18}F]DOPA or [^{11}C]DOPA are summarized in Table II. Six out of eight studies reported increased accumulation of DOPA in the striatum of patients with schizophrenia (Dao-Castellana *et al.*, 1997; Elkashef *et al.*, 2000; Hietala *et al.*, 1995, 1999; Lindstrom *et al.*, 1999; McGowan *et al.*, 2004; Meyer-Lindenberg *et al.*, 2002; Reith *et al.*, 1994), one reported no change (Dao-Castellana *et al.*, 1997) and one study reported reduced [^{18}F]DOPA striatal uptake (Elkashef *et al.*, 2000). Three studies involved first-episode schizophrenia, and all three showed an increase of DOPA in the striatum (Hietala *et al.*, 1995, 1999; Lindstrom *et al.*, 1999). Interestingly, a recent study observed a relationship between poor prefrontal activation during the Wisconsin Card Sorting task and elevated [^{18}F]DOPA accumulation in the striatum, suggesting a link between alteration of the dorsolateral PFC function and increased striatal DA activity in schizophrenia (Meyer-Lindenberg *et al.*, 2002). In rats as in anesthetized pigs, increases in aromatic L-amino acid decorboxylase (AADC) activity *in vitro* and *in vivo* have been reported following acute treatment with DA antagonists (Cho *et al.*, 1997; Danielsen *et al.*, 2001; Zhu *et al.*, 1993). Conversely, acute treatment with the DA agonist apomorphine decreases ^{11}C-DOPA influx in monkeys (Torstenson *et al.*, 1998). Evidence for such effects in humans, however, is extremely limited. Thus, in the only comprehensive study to date Grunder *et al.* (2003) reported a decrease in [^{18}F]DOPA uptake in nine

patients with schizophrenia following subchronic treatment with haloperidol, suggesting that chronic neuroleptic administration will tend to decrease AADC activity and hence DA synthesis. Interestingly, acute administration of antipsychotics increases DA neurons firing whereas chronic administration decreases the number of spontaneously active DA neurons in the rat SN (Grace, 1991), suggesting that the different effects of antipsychotics on AADC activity in the living brain could reflect such phenomena.

2. *Prefrontal DA Function and Schizophrenia*

Indirect evidence supports the hypothesis that a deficit in prefrontal DA function might contribute to prefrontal impairment in schizophrenia. Abundant preclinical evidences have documented the importance of prefrontal DA function for cognition (for review see Goldman-Rakic, 1994; Goldman-Rakic *et al.*, 2000). This important role has been recently confirmed in humans, by the repeated observation that the carriers of the high-activity allele of cathecol-*O*-methyltransferase (COMT), an enzyme involved in DA metabolism, display lower performance in various cognitive tasks compared to carriers of the allele that induces lower concentration of DA in PFC (for review see Goldberg and Weinberger, 2004). Clinical studies have suggested a relationship between low cerebrospinal fluid homovanillic acid, a measure reflecting low DA activity in the PFC, and poor performance at tasks involving WM in schizophrenia (Kahn *et al.*, 1994; Weinberger *et al.*, 1988). Administration of DA agonists might have beneficial effects on the pattern of prefrontal activation measured with PET during these tasks (Daniel *et al.*, 1991; Dolan *et al.*, 1995). While these observations are consistent with the hypothesis of a hypodopaminergic state in the PFC of patients with schizophrenia, they do not constitute direct evidence.

The only parameter of DA transmission that is currently quantifiable using noninvasive *in vivo* studies is D1 receptor availability. Three PET studies of prefrontal D1 receptor availability in patients with schizophrenia have recently been published. Two studies were performed with [^{11}C]SCH 23390. The first reported decreased [^{11}C]SCH 23390 BP in the PFC (Okubo *et al.*, 1997) and the other reported no change (Karlsson *et al.*, 2002). One study was performed with [^{11}C]NNC 112 (Abi-Dargham *et al.*, 2002) and reported increased [^{11}C]NNC 112 BP in the DLPFC and no change in other regions of the PFC such as the medial prefrontal cortex (MPFC) or the orbitofrontal cortex. In patients with schizophrenia, increased [^{11}C]NNC 112 binding in the DLPFC was predictive of poor performance on a working memory task (Fig. 5; Abi-Dargham *et al.*, 2002). Many potential factors including patient heterogeneity and differences in the boundaries of the sampled regions might account for these discrepancies. However, severity of deficits at tasks involving WM were reported to be associated with both decreased

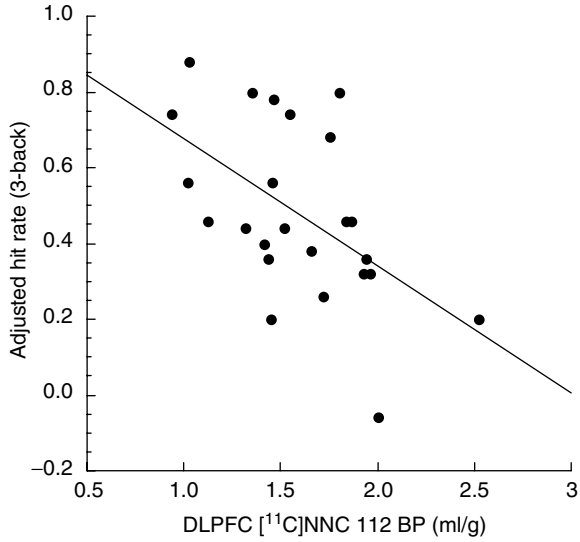

FIG. 5. Relationship between upregulation of D1 receptors in the DLPFC of untreated patients with schizophrenia and performance at WM task (3-back adjusted hit rate or AHR, lower values represent poorer performance).

PFC [^{11}C]SCH 23390 BP in one study (Okubo *et al.*, 1997) and increased PFC [^{11}C] NNC 112 BP in another one (Abi-Dargham *et al.*, 2000a), suggesting that both alterations might reflect a common underlying deficit.

Because of the prevalent view that schizophrenia is associated with a deficit in prefrontal DA activity, the impact of acute and subchronic DA depletion on the *in vivo* binding of [^{11}C]SCH 23390 and [^{11}C]NNC 112 is highly relevant to the interpretation of these data (Guo *et al.*, 2001). Acute DA depletion does not affect the *in vivo* binding of [^{11}C]NNC 112, but results in decreased *in vivo* binding of [^{3}H]SCH 23390, a paradoxical response that might be related to DA depletion-induced translocation of D1 receptors from the cytoplasmic to cell surface compartment (Dumartin *et al.*, 2000; Laruelle, 2000a; Scott *et al.*, 2002). In contrast, chronic DA depletion is associated with increased *in vivo* [^{11}C]NNC 112 binding, presumably reflecting a compensatory upregulation of D1 receptors. Interestingly, chronic DA depletion did not result in enhanced *in vivo* binding of [^{3}H]SCH 23390, an observation maybe related to opposite effects of receptors upregulation and externalization.

Thus, the increase in DLPFC [^{11}C]NNC 112 BP observed in schizophrenia might be related to a compensatory but inefficient upregulation of D1 receptors following sustained DA depletion, and it is conceivable that such an upregulation might not be detectable with [^{11}C]SCH 23390. Studies with both radiotracers on the same patients are required to clarify this issue.

IV. Conclusions

The development of new imaging methods aiming at measuring presynaptic activity in striatal DA afferents provided data consistent with the view that schizophrenia is associated with hyperactivity of subcortical transmission at D2 receptors (Fig. 6). These results are consistent with the known mode of action

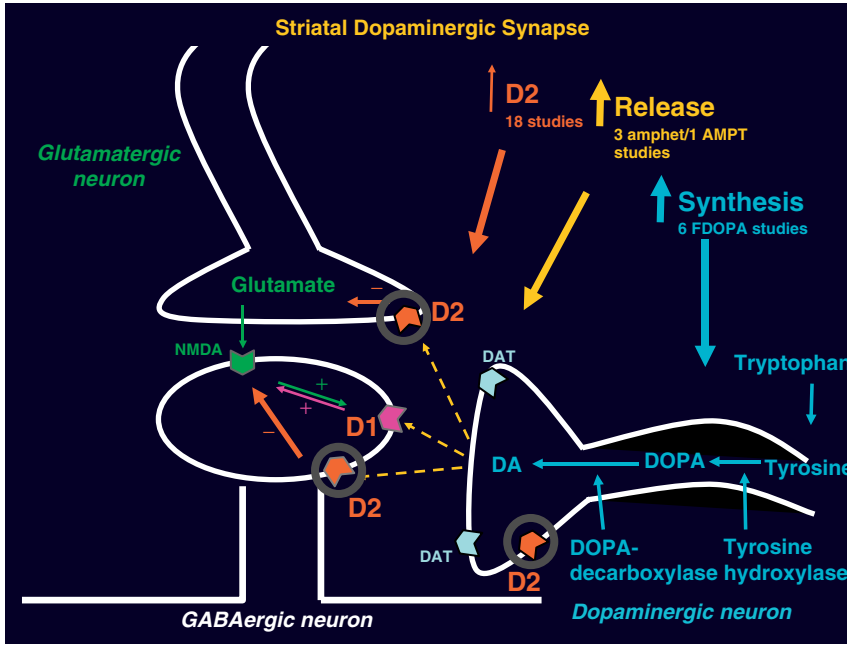

FIG. 6. Striatal dopaminergic synapse, summary of findings: evidence for excess DA transmission derives from pre- and postsynaptic studies. Excess DA transmission may impair glutamatergic NMDA transmission by a D2-mediated impaired presynaptic release of glutamate and an imbalance of D1/D2 opposing effects onto NMDA transmission. See text and tables for references.

of current antipsychotic treatment (D2 receptor blockade), with the psychotogenic effects of sustained stimulation of DA function by psychostimulants, and with the "classical" DA hypothesis of schizophrenia derived from these observations. In addition, these results suggest that the DA hyperactivity of subcortical systems is episodic in nature, and account for only some aspects of positive symptomatology. On the other hand, imaging methods might suggest that hypodopaminergia in the DLPFC participate to do pathophysiology of cognitive symptoms endured by patients with schizophrenia.

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THE DOPAMINE SYSTEM AND THE PATHOPHYSIOLOGY OF SCHIZOPHRENIA: A BASIC SCIENCE PERSPECTIVE

Yukiori Goto*,†,‡ and Anthony A. Grace*,†,‡

*Department of Neuroscience, University of Pittsburgh, Pittsburgh, Pennsylvania 15260, USA
†Department of Psychology, University of Pittsburgh, Pittsburgh, Pennsylvania 15260, USA
‡Department of Psychiatry, University of Pittsburgh, Pittsburgh, Pennsylvania 15260, USA

The dopamine system has been a subject of intense investigation due to its role in a number of normal functions and its disruption in pathological conditions. Thus, the dopamine system has been shown to play a major role in cognitive, affective, and motor functions, and its disruption has been proposed to underlie the pathophysiology of several major psychiatric and neurological disorders, including schizophrenia, Parkinson's disease, drug abuse, and attention deficit/hyperactivity disorder. Although these studies have continued to define the basic functional principles of the dopamine system in the mammalian brain, we are still at the initial stages in unraveling the complex role of this transmitter system in regulating behavioral processes. Accumulating evidence suggests that dopamine modulates excitatory and inhibitory neurotransmission, and moreover affects synaptic plasticity induced within the circuits of its target brain regions. It is this role in synaptic plasticity that has associated the dopamine system with aspects of cognitive function involving learning and memory. In this chapter, we summarize recent findings relevant to the role of the dopamine system in psychiatric disorders at cellular, anatomical, and functional levels. In particular, we will focus on the regulation of dopamine neuron activity states and how this impacts dopamine release in cortical and subcortical systems, and the physiological and behavioral impact of dopamine receptor stimulation in the postsynaptic targets of these neurons. A brief summary of recent findings regarding the development and maturation of DA

INTERNATIONAL REVIEW OF NEUROBIOLOGY, VOL. 78
DOI: 10.1016/S0074-7742(06)78002-3

0074-7742/07 $35.00

system and how this relates to the pathophysiology of psychiatric disorders are given, and finally models of dopamine system disruption in schizophrenia and how therapeutic approaches impact on dopamine system dynamics is presented.

I. Introduction

Dopamine (DA) is the most basic of the catecholamine neurotransmitters in the central nervous system. Since the identification of DA as an independent neurotransmitter in the brain (Carlsson, 1959; Carlsson *et al.*, 1958), a large number of studies from molecular to behavioral levels has been done to understand the functional roles of DA. However, there is general agreement from research to suggest that the role of DA is not to mediate direct synaptic driving of neurotransmission in the brain, but instead to modulate excitatory and inhibitory neurotransmission (Kupfermann, 1979). Therefore, DA is now considered to be a neuromodulator.

The reason for this substantial level of interest is due to its involvement in a number of neurological and psychiatric disorders including Parkinson's disease (Hornykiewicz, 1971; Lloyd and Hornykiewicz, 1970) and schizophrenia (Faurbye, 1968; Fischer, 1970). The loss of DA neurons in the nigrostriatal system has been shown to underlie the symptoms of Parkinson's disease (Hornykiewicz, 1971; Lloyd and Hornykiewicz, 1970), whereas excessive DA release has been suggested in the pathophysiology of schizophrenia. The latter is based on studies showing that DA agonists such as amphetamine induce psychosis similar to that of schizophrenia patients (Connell, 1958; Snyder, 1972) as well as the fact that antipsychotic drugs used for the treatment of schizophrenia are DA receptor antagonists (Carlsson, 1974; Seeman, 1987). These studies suggest that proper DA signaling in the brain is critical for cognitive and affective functions as well as motor control, which are disrupted in these neurological and psychiatric disorders.

In this chapter, we summarize recent findings of the DA system at cellular, pharmacological, physiological, and functional levels, and how these findings relate DA system function to the pathophysiology of schizophrenia.

II. Neuroanatomy of DA Systems

DA neurons are located in the mesencephalon and project into the forebrain along three major pathways.

The nigrostriatal system is composed of DA neurons located in the substantia nigra pars compacta (SNc) that project into the dorsal striatum (Anden *et al.*, 1964;

Bedard *et al.*, 1969). This is the system that is believed to have a principal involvement in motor control, since degeneration of DA neurons in the SNc is the primary pathology of Parkinson's disease (Hornykiewicz, 1971; Lloyd and Hornykiewicz, 1970). Of particular importance in the functional significance of nigrostriatal DA projections is its involvement in motor learning and habit formation (Graybiel, 1998; Jog *et al.*, 1999). However, animal and human studies have also revealed that the nigrostriatal DA projection is also involved in non-motor cognitive functions (Carbon and Marie, 2003; Graybiel, 1997; Schultz, 2002).

The mesolimbic DA system is composed of DA neurons located in the ventral tegmental area (VTA) that project to the ventral striatum (VS) including the nucleus accumbens, olfactory tubercle, and limbic structures such as the basolateral amygdala and hippocampus (HPC) (Brinley-Reed and McDonald, 1999; Fallon and Moore, 1978; Fallon *et al.*, 1978; Scatton *et al.*, 1980; Voorn *et al.*, 1986). DA release in the amygdala and HPC are thought to be involved in emotional learning (Bissiere *et al.*, 2003; Jellestad *et al.*, 1986; Rosenkranz and Grace, 2002) and long-term memory (Li *et al.*, 2003; Lisman and Grace, 2005; Otmakhova and Lisman, 1996), respectively. The VS is the brain region where limbic and cortical inputs converge, and information encoded on these brain structures is integrated to organize goal-directed behavior (Groenewegen *et al.*, 1996; Mogenson *et al.*, 1980). As such, the mesolimbic DA projections to the VS modulate this information integration, and thereby influence motor behavior. In particular, these DA projections into the VS are considered to be crucial for motivation and reward seeking (Everitt and Robbins, 2005; Iversen, 1984).

Finally, the mesocortical DA system is composed of DA neurons also located in the VTA that project to the prefrontal cortex (PFC), anterior cingulate, and entorhinal cortex in rodents (Fallon *et al.*, 1978; Thierry *et al.*, 1973) as well as additional neocortical and even cerebellar areas in primates and humans (De Keyser *et al.*, 1989; Lewis *et al.*, 1987; Melchitzky and Lewis, 2000; Moore *et al.*, 2003). The PFC is considered to be the highest center of cognition (Funahashi, 2001; Fuster, 1997; Goldman-Rakic, 1995; Knight *et al.*, 1995; Robbins, 2000; Shimamura, 2000), and DA release in the PFC is essential for its function. As such, a number of cognitive functions such as short-term memory (Funahashi *et al.*, 1993; Goldman-Rakic, 1995), attention (Gorenstein *et al.*, 1989; Knight *et al.*, 1995; Muir *et al.*, 1996), future planning (Baker *et al.*, 1996; Ingvar, 1985; Owen *et al.*, 1990), and set shifting (Milner, 1963; Owen *et al.*, 1993) have been proposed to be mediated by the PFC, and interruption of the mesocortical DA innervation of the PFC is known to produce impairments in these functions (Floresco *et al.*, 2006; Ragozzino, 2002; Sawaguchi and Goldman-Rakic, 1994; Seamans *et al.*, 1998).

The VTA is a heterogeneous structure consisting of DA neurons and gamma-aminobutyric acid (GABA) neurons. Some of these GABA neurons are interneurons, whereas other GABA neurons are projection neurons that innervate both the

PFC and VS. Anatomical and physiological studies (Carr and Sesack, 2000; Floresco *et al.*, 2001, 2003; Lisman and Grace, 2005; Sesack and Carr, 2002) have shown that the mesolimbic and mesocortical DA pathways are organized into two independent closed loops.

In the mesocortical DA system, PFC pyramidal neurons (in layers V and VI) are reported to project selectively onto VTA DA neurons that project back to the PFC (Fig. 1; Carr and Sesack, 2000; Sesack and Carr, 2002). In addition, PFC afferents are also shown to target GABA interneurons that in turn regulate the activity of DA neurons that project to the VS as well as GABA neurons that project directly to the VS (Fig. 1; Carr and Sesack, 2000; Sesack and Carr, 2002). Consequently, this anatomically closed loop induces higher DA release in the PFC during periods of PFC activation, while at the same time it suppresses DA release in the VS.

In contrast, the mesolimbic DA system also forms another closed loop circuit with the HPC and VS (Fig. 2). Thus, the HPC send excitatory projections into the VS (Groenewegen *et al.*, 1987; Kelley and Domesick, 1982), which in turn regulates other basal ganglia nuclei including the ventral pallidum and pedunculopontine tegmentum (Floresco *et al.*, 2003). Through this loop, activity within the HPC efferents is positioned to control DA release in the VS (Floresco *et al.*, 2001).

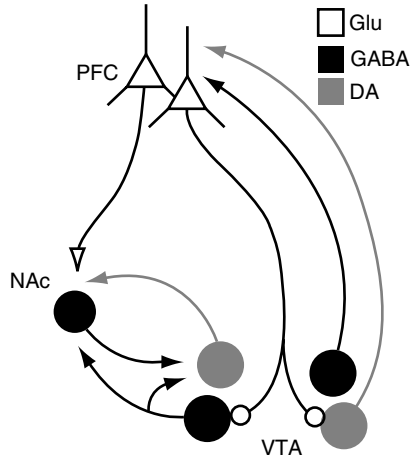

FIG. 1. Schematic diagram of the relationship between the PFC, VS, and the DA system. The reciprocal interaction between the PFC and VTA would allow PFC activation to selectively induce DA release in the PFC and suppress DA release in the VS simultaneously. Glu and NAc denote glutamate and nucleus accumbens, respectively. Reprinted from Sesack and Carr, copyright (2002), with permission from Elsevier.

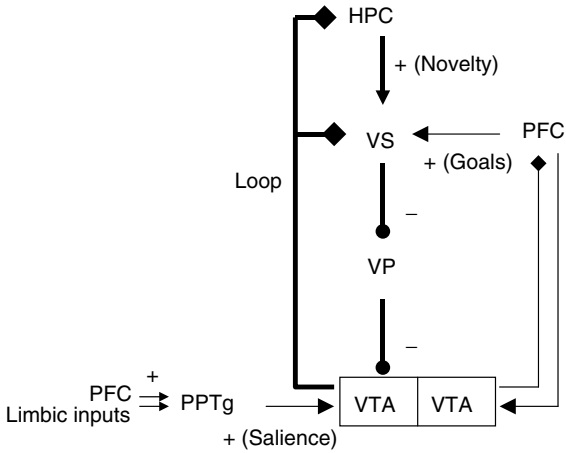

FIG. 2. Functional interactions between the HPC and the VTA that can mediate novelty detection and incorporation into memory. It is proposed that the incorporation of an object or an event into memory is dependent on two aspects: novelty and salience. In this model, HPC afferents regulate activity within the VS and its afferent basal ganglia nuclei including the ventral pallidum (VP). These structures, in turn, can modulate VTA DA neuron activity. It is proposed that novelty detection in the HPC will cause excitation of VTA DA neurons via this pathway. When the HPC novelty-dependent excitation of the VTA occurs in coincidence with salience-dependent activation of pedunculopontine tegmental (PPTg) input to the VTA neurons, the resultant enhanced VTA DA input to the HPC will trigger incorporation into memory. Triangles, circles, and diamonds indicate glutamate, GABA, and DA projections, respectively. Adapted from Lisman and Grace (2005).

III. DA Neuron Activity and Release

DA neurons are known to exhibit two types of spike firing patterns: tonic and burst spike firing (Grace and Bunney, 1984a,b). Tonic spike firing is the baseline spontaneous activity state that is driven by an endogenous pacemaker conductance (Grace and Bunney, 1984b) and does not depend on an excitatory driving force. Thus, tonic spike firing is still observed in an *in vitro* brain slice preparation in which the afferent inputs onto the DA neurons are transected. In contrast, transient, burst spike firing is known to be triggered by external stimuli, especially those associated with the presentation of unexpected reward or sensory signals that predict rewards (Schultz *et al.*, 1993). Furthermore, DA neurons exhibit transient suppression of tonic spike firing with aversive stimuli or omission of expected rewards (Tobler *et al.*, 2003; Ungless *et al.*, 2004). However, it is still controversial regarding whether only reward-associated stimuli can evoke burst spike firing in DA neurons (e.g., burst spike firing evoked by aversive stimuli; Guarraci and Kapp, 1999; Mantz *et al.*, 1989). Phasic burst spike firing is regulated

by excitatory glutamatergic inputs onto DA neurons arising from other brain structures including the PFC (Au-Young *et al.*, 1999; Gariano and Groves, 1988), subthalamic nucleus (Chergui *et al.*, 1994; Smith and Grace, 1992), and glutamatergic/cholinergic afferents from the pedunculopontine tegmentum onto DA neurons (Floresco *et al.*, 2003; Futami *et al.*, 1995). Furthermore, the ability of DA neurons to fire in bursts is dependent on inputs from the lateral dorsal tegmental nucleus (Lodge and Grace, 2006a); interruption of this source of afferents prevents glutamate-driven burst firing, causing the DA neurons to discharge in a manner similar to that observed *in vitro* (Grace and Onn, 1989).

DA is released from DA terminals located in the targeted brain areas as a function of spike firing patterns of DA neurons (Fig. 3). Tonic spike firing of DA neurons induces tonic, low concentration of DA release (i.e., nanomolar; Floresco *et al.*, 2003; Grace, 1991). *In vivo*, the level of tonic DA release is regulated by powerful GABAergic inhibitory inputs arising from the ventral pallidum that innervate DA neurons in the VTA. Thus, when the ventral pallidum is inactivated, the number of spontaneously firing DA neurons increases and tonic DA release in the VS is increased (Floresco *et al.*, 2003). It is hypothesized that such tonic DA release can escape from the reuptake system in the synaptic cleft of the release sites, and therefore determines the basal concentration of extracellular DA level within these regions (Fig. 3; Grace, 1991). Tonic DA release is too low in concentration to effectively stimulate the postsynaptic targets of these neurons; however, it appears to be sufficient in concentration to stimulate presynaptic DA receptors, including those on corticostriatal projections (O'Donnell and Grace, 1994; West and Grace, 2002) and the autoreceptors on the DA terminals themselves, which provide a local regulation of DA synthesis and release (Nowycky and Roth, 1978; Starke *et al.*, 1978; Wolf and Roth, 1990). In contrast, burst spike firing of DA neurons induces a substantially higher amplitude (i.e., micromolar to millimolar; Floresco *et al.*, 2003; Grace, 1991) of phasic DA release within the synaptic cleft of the DA terminals, where it can stimulate postsynaptic DA receptors. This high-amplitude phasically released DA is then subject to immediate reuptake into DA terminals via the dopamine transporter (DAT) in the striatum, where it is then recycled or metabolized by monoamine oxidase (Fig. 3; Grace, 1991; Kuhar *et al.*, 1990; Molinoff and Axelrod, 1971). This contrasts with the PFC, in which DAT is low, and DA is believed to be removed primarily by actions of metabolic enzyme catechol-*O*-methyltransferease (COMT) (Fig. 3; Karoum *et al.*, 1994; Molinoff and Axelrod, 1971; Sesack *et al.*, 1998; White, 1996) or by reuptake into noradrenergic terminals (Moron *et al.*, 2002). Indeed, DAT knockout mice show a greater DA overflow in the striatum and hyperlocomotion, but the amount of DA reuptake is not changed in the PFC of these mice (Gogos *et al.*, 1998). Recent studies combining human imaging studies with genetic analysis have revealed that single nucleotide polymorphism (SNP) of genes encoding COMT affects PFC function. For example, human subjects with the Val/Val allele at position 158 in COMT exhibit significantly

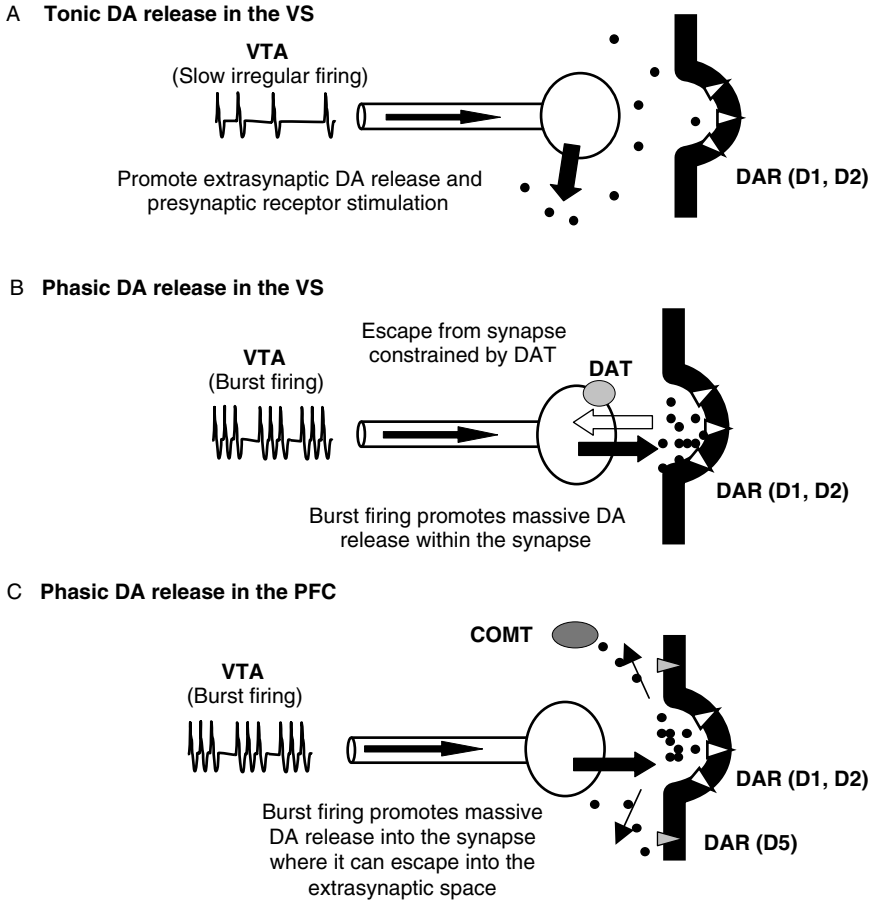

FIG. 3. Tonic and phasic DA release in the PFC and striatum is regulated by COMT and DAT. (A) The slow, irregular baseline spike firing of DA neurons induces steady-state tonic DA release in the postsynaptic target regions. DA released in this manner is not strongly attenuated by the reuptake system, and hence can diffuse into the extrasynaptic space. (B) When burst spike firing occurs in DA neurons, massive, phasic DA release is produced in the striatum. Phasic DA release is subject to rapid removal by the DA uptake system, DAT, before it can escape the synaptic cleft. Therefore, phasic DA release is transient and spatially constrained to the vicinity of the synaptic cleft. (C) In contrast, in the PFC where there are very low levels of DAT, the primary means for inactivation of released DA is via the metabolizing enzyme COMT. As a result, both tonic and phasic DA released from terminals in the PFC is allowed to diffuse into the extrasynaptic space. One of the targets that extrasynaptic DA can stimulate is the D5 receptor, which is selectively located in this compartment. Adapted from Bilder *et al.* (2004).

lower working memory and PFC activity than those having the Met/Met allele. Since Val/Val COMT metabolizes DA at a faster rate than Met/Met COMT, this difference is believed to affect DA dynamics within the PFC (Egan *et al.*, 2001; Weinberger *et al.*, 2001). Given that COMT cannot inactivate DA actions as efficiently as that produced by DAT in the subcortical area, it is likely that tonic and phasic DA release play substantially different roles between the PFC and the VS, with phasic DA release underlying extrasynaptic DA concentrations that are regulated by COMT activity (Fig. 3; Bilder *et al.*, 2004). The increased area of diffusion of extracellular DA in the PFC, in addition to affecting a number of neuronal elements, may also lead to a selective stimulation of a particular subtype of DA receptor. Thus, a study has shown that the DA D5 receptor subtype is located exclusively at extrasynaptic sites of PFC neurons (Paspalas and Goldman-Rakic, 2004). This would position the D5 receptor to respond preferentially to tonic DA that has diffused away from its release site.

Studies have found that tonic and phasic DA release can actually regulate the balance between PFC and limbic inputs into the VS (Goto and Grace, 2005b). It was found that inactivation of the ventral pallidum to disinhibit spontaneous DA neuron firing and consequently increase tonic DA release induces attenuation of PFC responses in the VS, which is also mimicked by local infusion of a D2 agonist. In contrast, activation of the ventral pallidum to decrease tonic DA release causes a selective increase of PFC responses in the VS, which is also mimicked by local infusion of a D2 antagonist. Therefore, as observed *in vitro* (O'Donnell and Grace, 1994) and *in vivo* (Brady and O'Donnell, 2004; West and Grace, 2002), the DA system exerts a bimodal regulation over the PFC afferents to the VS, and this modulation is controlled by tonic DA release caused by spontaneous DA neuron spike firing (Goto and Grace, 2005b). In contrast, when the DA neurons are caused to burst firing by activation of the pedunculopontine tegmental glutamatergic afferent drive to the VTA, the resultant phasic DA release was found to selectively augment HPC afferent drive of the VS without affecting PFC inputs. This action can be mimicked by local infusion of a D1 agonist. However, unlike the D2 system, there does not appear to be a baseline D1 modulation of HPC synaptic drive on VS neurons.

The information gleaned from the studies above shows that the tonic and phasic DA system can exert unique effects on afferent integration within the VS. However, it is likely that the tonic and phasic DA system work in concert to modulate VS function, rather than as independent entities. Indeed, we have found that these systems can function in an integrated manner to augment DA function. Thus, we have found that burst firing can only be induced in DA neurons that are exhibiting spontaneous spike firing. This is likely due to the fact that burst firing is dependent on N-methyl-D-asparate (NMDA) receptor stimulation (Overton and Clark, 1992, 1997), and in a hyperpolarized, nonfiring neuron, NMDA channels are not closed by a magnesium block (Mayer *et al.*, 1984; Nowak *et al.*, 1984). This

hyperpolarized state can be relieved by decreasing the massive bombardment of DA neurons by GABAergic inhibition (Grace and Bunney, 1979, 1985) that hold these neurons in the inactive state. When tonic DA spike firing is activated by a decrease in ventral pallidal inhibitory control, pedunculopontine tegmental glutamatergic inputs are capable of causing a much larger population of DA neurons to enter the burst firing mode (Lodge and Grace, 2006b). With the tonic and phasic system acting together, DA transmission in the VS would exert two effects: a tonic DA D2-mediated attenuation of PFC drive and a phasic D1-mediated potentiation of HPC drive over VS neurons. Thus, increasing DA transmission will shift the balance of inputs to the VS away from PFC drive, favoring limbic drive. Conversely, a decrease in DA system function, as may occur during cognitive states or as a consequence of antipsychotic drug-induced depolarization block as described below (Section VII; Grace *et al.*, 1997), would shift the balance toward PFC predominance over the limbic input.

IV. Cellular Actions of DA

There are two major classes of DA receptors that mediate the actions of DA: the D1 family and the D2 family. The D1 receptor family has two subclasses, D1 and D5, whereas D2 receptor family is composed of three subclasses, D2, D3, and D4 receptors (Jackson and Westlind-Danielsson, 1994; Seeman and Van Tol, 1993). These receptors are located at both pre- and postsynaptic sites. D1 and D2 receptors are coupled to Gs and Gi proteins and, as a consequence, mediate activation and inhibition of second messenger cascades, respectively (Greengard *et al.*, 1999; Neve *et al.*, 2004). This leads to phosphorylation and dephosphorylation of a number of channels and receptors, modulating cell excitability (Cepeda *et al.*, 1993, 1995; Gorelova and Yang, 2000; Gulledge and Jaffe, 1998; Tseng and O'Donnell, 2004; Wang and Goldman-Rakic, 2004), glutamate and GABA neurotransmission (Gao *et al.*, 2001; Seamans *et al.*, 2001a,b), and synaptic plasticity (Bissiere *et al.*, 2003; Centonze *et al.*, 2001; Otani *et al.*, 2003; Otmakhova and Lisman, 1996; see references such as Nicola *et al.*, 2000 and Seamans and Yang, 2004 for detailed summary of channels and receptors modulated by DA). The most consistent finding with respect to DA modulation of channels and receptors in the PFC and striatum is the D1-mediated facilitation of calcium influx into neurons via interactions with NMDA channels (Cepeda *et al.*, 1993; Tseng and O'Donnell, 2004) and L-type calcium (Surmeier *et al.*, 1995; Tseng and O'Donnell, 2004). In contrast, although it is still controversial, the D2 receptors have been suggested to decrease AMPA in the PFC and striatum (Cepeda *et al.*, 1993; Tseng and O'Donnell, 2004). DA also seems to increase or decrease GABA release via D1 and D2 receptors, respectively, located on interneurons

in the PFC (Gorelova *et al.*, 2002; Seamans *et al.*, 2001b). However, another study shows that D2 receptor activation has the opposite action: an increase in GABA release in the PFC (Tseng and O'Donnell, 2004). Anatomical studies have shown that D1 receptors are found on presynaptic sites of terminals within the PFC (Paspalas and Goldman-Rakic, 2005), and stimulation of presynaptic D1 receptors have been reported to decrease glutamate release in the PFC (Gao *et al.*, 2001; Seamans *et al.*, 2001a). In contrast, D1 receptors have not been found to be present presynaptically on terminals within the striatum (Hara and Pickel, 2005). Instead, in the striatum only the D2 DA receptor has been localized to presynaptic sites (Fisher *et al.*, 1994; Wang and Pickel, 2002). Although the role of presynaptic DA receptors in controlling glutamate and/or GABA release has not been fully characterized, within the striatum, stimulation of presynaptic D2 receptors has been shown to decrease glutamate release (Bamford *et al.*, 2004; Umemiya and Raymond, 1997) and to attenuate PFC-evoked excitatory postsynaptic potentials (EPSPs) in the striatum (Brady and O'Donnell, 2004; Goto and Grace, 2005b; O'Donnell and Grace, 1994; West and Grace, 2002).

It is difficult to examine the cellular effects of DA transmission *in vivo*, given the potent regulation of the system and the multiple sites of action of DA release. In order to examine how DA is modulating activity within the striatum, studies were conducted in which DA antagonists were infused locally into the striatum via reverse microdialysis to achieve local blockade of receptors while performing *in vivo* electrophysiological recordings from neurons adjacent to the probe, through which the effects produced by attenuating endogenous DA transmission can be observed (West and Grace, 2002). Using this methodology, it was found that striatal neurons *in vivo* are potently modulated by DA D1 and D2 receptors, with D1 receptors controlling neuronal excitability and D2 receptors modulating baseline activity and cortical afferent drive (West and Grace, 2002).

Functional studies into the significance of DA modulation of neuronal activity points to the involvement of DA in synaptic plasticity and learning and memory associated with it (Schultz, 2002). Synaptic plasticity has been most commonly tested by induction of long-term potentiation (LTP) and depression (LTD; Abraham and Tate, 1997; Bear and Malenka, 1994). Since LTP and LTD induction is closely linked with intracellular calcium influx (Abraham and Tate, 1997; Bear and Malenka, 1994), D1-mediated NMDA and L-type calcium channel phosphorylation have a critical impact on them (Greengard *et al.*, 1999). Indeed, synaptic plasticity induction in brain regions receiving DA innervation, including the PFC (Gurden *et al.*, 1999; Otani *et al.*, 1998), striatum (Arbuthnott *et al.*, 2000; Centonze *et al.*, 2001; Reynolds *et al.*, 2001), HPC (Li *et al.*, 2003; Otmakhova and Lisman, 1996), and amygdala (Bissiere *et al.*, 2003), is dependent on DA release. For example, D1 antagonist is known to prevent LTP induced at HPC afferents into the PFC (Gurden *et al.*, 2000).

Furthermore, LTD induction within PFC circuitry is shown to depend on both D1 and D2 receptor activation (Otani *et al.*, 1998). Similarly, D1- but not D2-dependent LTP has been found in the HPC (Otmakhova and Lisman, 1996) and amygdala (Bissiere *et al.*, 2003). However, LTP and LTD induction in the striatum is somewhat controversial. DA-dependent plasticity that involves both D1 and D2 receptors has been reported with respect to LTP and LTD induction in the dorsal striatum (Calabresi *et al.*, 1992; Spencer and Murphy, 2000), whereas despite the presence of dense DA projections, synaptic plasticity in the VS has been reported to be DA independent (Pennartz *et al.*, 1993). In contrast, studies have described DA-dependent plasticity in the VS (Goto and Grace, 2005a; Thomas *et al.*, 2001). Some studies have shown that DA affects NMDA and AMPA receptor trafficking (Malenka, 2003; Wolf *et al.*, 2004). D1 receptor activation stimulates cell surface expression of GluR1 subunit of NMDA receptors in the PFC (Sun *et al.*, 2005) and VS (Mangiavacchi and Wolf, 2004), resulting in increased calcium influx into the neurons.

Overall, DA appears to control the balance of excitatory and inhibitory drive of neural activity within its projection sites, and to mediate synaptic plasticity associated with learning and memory.

V. Roles of DA on Cognitive and Affective Functions

DA is involved in the induction of synaptic plasticity in the brain regions receiving DA neuron projections, and therefore is believed to play a pivotal role in learning and memory processes (Schultz, 2002). An elegant study of electrophysiological recordings from DA neurons in primates done by Schultz and colleagues (Waelti *et al.*, 2001) have revealed that the activation of DA neuron spike discharge during learning trials is consistent with that predicted by learning theory (Rescorla–Wagner rule of classical conditioning; Rescorla and Wagner, 1972), suggesting that DA signals can be used to prime selective brain areas for learning (Schultz, 2002). DA signals are used to mediate different types of learning and memory depending on the specific regions involved (Schultz *et al.*, 2000, 2003). Thus, DA projections into the dorsal striatum are critical for motor learning and habit formation such as playing musical instruments or riding a bicycle (Graybiel, 1998). Moreover, studies have shown that the DA innervation of limbic structures plays a different role in mediating learning processes. Thus, the DA innervation of the HPC appears to be involved in the formation of long-term memory (Lisman and Grace, 2005), whereas DA projections into the amygdala mediate emotional memory such as aversive conditioning (Nader and LeDoux, 1999; Rodrigues *et al.*, 2004; Rosenkranz and Grace, 2002). The role of the DA innervation of the VS in learning processes is less clear. However,

the VS is the brain region where limbic and cortical inputs converge to integrate context- and emotion-associated goal-directed behavior (Mogenson *et al.*, 1980). Therefore, it is likely that mesolimbic DA projections to the VS are involved in learning processes that could occur in acquiring these aspects of goal-directed motor control (Everitt and Robbins, 2005; Kelley and Berridge, 2002). Given that the DA system is also believed to provide a "learning signal" to the limbic system (Schultz, 2002), we examined the ability of DA transmission to exert short-term modulation of the PFC and HPC drive within the VS, and how this relates to the processing of goal-directed behavior (Goto and Grace, 2005b). Disconnection of the HPC inputs from the VS by unilateral injection of lidocaine into the HPC while infusing D1 antagonists into the contralateral VS was found to interfere with the efficient acquisition of goal-directed behavior. In contrast, when the PFC was disconnected from the VS by unilateral infusion of lidocaine into the PFC while injecting a D2 agonist into the contralateral VS, there was no interference in the acquisition of an initial discrimination task. Instead, when the response strategy was altered (i.e., when the rat had to change from a visually guided response strategy to a direction-guided response strategy), the rats exhibited perseveration, using the previous response strategy rather than switching to the new paradigm. This suggests that D1-dependent HPC-VS information processing mediates learning of a response strategy, whereas D2-dependent PFC-VS information processing is crucial for flexible switching of this response strategy in guiding goal-directed behavior (Goto and Grace, 2005b). This type of disruption of limbic and cortical balance in regulating information processing in the VS for goal-directed behavior is consistent with the type of behavioral disruption proposed to occur in drug addiction (Everitt and Wolf, 2002; Kelley and Berridge, 2002; Ridley, 1994). Thus, in drug-addicted subjects, a loss of cortically driven behavioral flexibility in favor of HPC-driven perseveration in drug-seeking behavior could be one of the mechanisms by which drug-addicted individuals lose their ability to modify their behavior in favor of more behaviorally effective strategies. Indeed, we also found that repeated cocaine treatment could induce abnormal strengthening of HPC inputs, with LTP induction in the HPC inputs and LTD attenuation of PFC inputs, resulting in an imbalance of limbic and cortical drive of VS activity (Goto and Grace, 2005a).

In contrast, the role of the mesocortical DA innervation into the PFC for learning and memory is not readily apparent from what we know about this system. Thus, the functions associated with the PFC, such as short-term storage of memory (few seconds to up to minutes; Funahashi *et al.*, 1993; Goldman-Rakic, 1995), flexible switching of response strategy (Milner, 1963; Owen *et al.*, 1993), attention (Gorenstein *et al.*, 1989; Knight *et al.*, 1995; Muir *et al.*, 1996), and future planning (Baker *et al.*, 1996; Ingvar, 1985; Owen *et al.*, 1990), are considered to be independent of what has been typically associated with more standard learning and memory paradigms, and may not involve synaptic plasticity

such as LTP and LTD induction in this region. Nevertheless, the PFC is known to exhibit synaptic plasticity in its network (Herry and Garcia, 2002; Laroche *et al.*, 1990; Otani *et al.*, 2003), and mesocortical DA release is essential for induction of such synaptic plasticity (Gurden *et al.*, 1999; Otani *et al.*, 1998), suggesting that any cognitive functions requiring proper DA release in the PFC could involve DA-dependent synaptic plasticity. However, the exact roles of such synaptic plasticity in the PFC have yet to be determined.

With respect to working memory, it has been reported that DA effects within the PFC are bimodal in function. Thus, there is an optimal level of DA stimulation required for proper PFC functioning, with either over- or understimulation of D1 receptors leading to dysfunctional states (Granon *et al.*, 2000; Zahrt *et al.*, 1997). This is an example of the classic "inverted U"-shaped relationship known as the Yerkes–Dodson curve (Yerkes and Dodson, 1908). In primate studies, it has been shown that D1 receptors play a crucial role in short-term memory functions (Goldman-Rakic, 1995). This is particularly pertinent for spatial working memory. Thus, when a monkey is required to hold the location of an object in memory for a short period of time in order to guide a subsequent behavior, PFC neurons associated with that special location become activated, and remain so until the task is completed (Funahashi *et al.*, 1993). Such sustained spike firing of PFC neurons during the time in which information is held in memory is disrupted by a D1 receptor antagonist (Sawaguchi and Goldman-Rakic, 1994). Whether D2 receptors also exhibit a U-shaped functional relationship in the PFC is not known. In primate electrophysiological recording studies, evidence for an involvement of D2 receptors in the temporal retention of information during short time periods has not been reported (Sawaguchi and Goldman-Rakic, 1994; Wang *et al.*, 2004). Nonetheless, evidence suggests that short-term memory can be affected by administration of D2 agonists or antagonists into human subjects (Kimberg *et al.*, 1997; Mehta *et al.*, 2001). Although this supports a D2 receptor involvement in short-term memory in humans, the location of this D2 action is not known.

VI. Development and Maturation of the DA System

There is increasing evidence that many major psychiatric disorders have their origin in a disruption occurring during development of the nervous system. Therefore, understanding the development and maturation of DA systems is essential for a more complete comprehension of the etiology and pathophysiology of a number of major psychiatric disorders such as schizophrenia and attention deficit/hyperactivity disorder (ADHD) in which neurodevelopmental compromises in the DA system have been implicated (Castellanos, 1997; Eells, 2003;

Harrison, 1999; Heyman and Murray, 1992; Nieoullon, 2002; Sonuga-Barke, 2005; Weinberger, 1987).

DA neurons are born in the midbrain of rats at around embryonic day (ED) 12–16, with a peak occurring at ED13 (Lauder and Bloom, 1974). This is followed by programmed cell death of subsets of DA neurons that is initiated around postnatal day (PD) 2 and PD14 (Jackson-Lewis *et al.*, 2000; Oo and Burke, 1997). Studies have shown that growth factors released in the areas targeted by DA neuron terminals such as the striatum appear to regulate this programmed cell death (Breiter *et al.*, 1997; Hyman *et al.*, 1991; Poulsen *et al.*, 1994). This pruning of the DA cell population in the midbrain continues to occur until about PD20 to arrive at the final adult population of midbrain DA neurons (Jackson-Lewis *et al.*, 2000; Oo and Burke, 1997). In contrast, the DA collateralization into the targeted areas continues to increase until adolescence begins. This occurs in concert with an increase in the number of DA receptors expressed in postsynaptic target areas. During puberty, a second wave of pruning of the DA innervation is initiated, but DA receptor pruning occurs differentially depending on the target region. Thus, there is substantial pruning of both D1 and D2 receptors in the PFC (Andersen *et al.*, 2000) and dorsal striatum (Teicher *et al.*, 1995) during adolescence and young adulthood. Indeed, it has been shown that the maturation of the DA system has functional significance with respect to D1-mediated facilitation of NMDA currents (Tseng and O'Donnell, 2004) and D2 receptor modulation of interneuron activity (Tseng and O'Donnell, 2004) in the PFC. In contrast, the reorganization of DA receptor expression in the VS during adolescence is not as prominent (Teicher *et al.*, 1995). Nevertheless, it appears that the mesolimbic DA system in the VS does not seem to be fully matured after puberty, since for example, drug sensitization produced by repeated psychostimulant administration induces significantly different effects in pre- and midpubertal animals (Tirelli *et al.*, 2003; Ujike *et al.*, 1995); a process that in the adult animal is known to involve sensitization of DA release in the VS (but see Borgland *et al.*, 2006 for evidence of sensitization in prepubertal animals). DA system maturation during adolescence is of particular interest with respect to the pathophysiology of schizophrenia, given that brain compromises at the second trimester of pregnancy have been suggested to occur in the brains of schizophrenia patients, whereas the onset of psychotic symptoms are typically delayed until late adolescence to early adulthood (Harrison, 1999; Weinberger, 1987). With respect to disorders such as ADHD, a similar type of disruption of mesocortical DA system function has also been proposed (Castellanos, 1997; Heyman and Murray, 1992; Nieoullon, 2002; Sonuga-Barke, 2005). However, the time course underlying the origin of ADHD symptoms appears to be substantially different from that of schizophrenia, since in schizophrenia the DA deficit occurs during maturation, whereas in ADHD the symptomatology is already present at a very early age.

VII. DA Deficits in Schizophrenia

Since the first description of amphetamine induction of schizophrenia-like symptoms in normals (Connell, 1958) and the observation that DA D2 antagonists are effective in the treatment of this disorder (Carlsson, 1974), a dysfunction within the DA system has been implicated in schizophrenia (Seeman, 1987). These classical studies suggested that schizophrenia symptoms may be caused by an excess in DA release; however, more recent studies suggest that this may be an oversimplification. Thus, results show that there is an augmentation of DA release in the striatum only during specific types of system activation, and this is correlated with the positive psychotic symptoms of this disorder (Laruelle *et al.*, 1999). In contrast, it has been proposed that the functional deficits observed in the PFC of schizophrenia patients could be due to a deficit in DA activity, which may underlie the negative or deficit state of schizophrenia (Abi-Dargham *et al.*, 2002; Davis *et al.*, 1991).

A potential relationship that has been advanced to account for the disturbed DA system in schizophrenia is an opposing relationship between PFC and striatal DA release (Fig. 4). Thus, primate (Kolachana *et al.*, 1995; Saunders *et al.*, 1998), rodent (Jackson *et al.*, 2001), and human studies (Meyer-Lindenberg *et al.*, 2002) have shown that the attenuated PFC activity in schizophrenia may be correlated with an exaggerated DA release in the striatum. This can be accounted for by an examination of the anatomical organization of PFC-VTA and HPC-VTA loops (Figs. 1 and 2; Lisman and Grace, 2005; Sesack and Carr, 2002). PFC projections onto GABA interneurons in the VTA can suppress spike firing in the DA neurons that project into the striatum. Therefore, with PFC deficits, the PFC drive of VTA DA neurons is diminished, leading to abnormally augmented DA release in the striatum in schizophrenia. Our study has revealed that increasing DA release in the VS facilitates limbic inputs and attenuates PFC inputs, whereas decreasing DA release shifts the balance in favor of the PFC inputs (Goto and Grace, 2005b), suggesting that DA maintains the balance between limbic and PFC drive of VS neurons. As such, a combination of abnormally attenuated PFC drive of VS neurons with augmented DA release in the VS that is shown to occur in schizophrenia patients (Laruelle *et al.*, 1999; Meyer-Lindenberg *et al.*, 2002) could cause inappropriate limbic drive of the VS and disruption of goal-directed behavior. A key mechanism for the inverse relationship between PFC activity and striatal DA release may be drawn from the known reciprocal interactions between the HPC and PFC (Fig. 4). Thus, the HPC sends direct projections into the PFC (Fuster, 1997; Jay *et al.*, 1989), whereas the PFC sends indirect projections into the HPC through the temporal cortex (Fuster, 1997; Groenewegen and Uylings, 2000; Kyd and Bilkey, 2003). Since it has been suggested that the PFC exerts an inhibitory influence over limbic structures (Fuster, 1997; Grace and Rosenkranz, 2002;

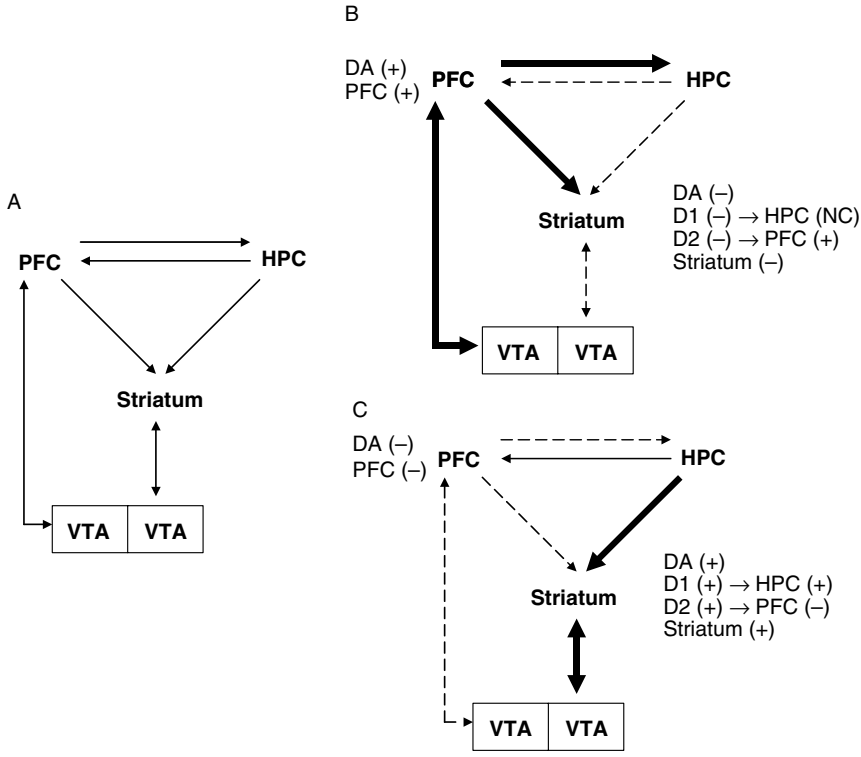

FIG. 4. Schematic diagrams of the mesocortical and mesolimbic DA system and its regulation by interactions among the PFC, HPC, and striatum. (A) The reciprocal interaction between the PFC and HPC maintains the balance of mesocortical and mesolimbic DA release. (B) When PFC activity is stronger than HPC activity, mesocortical DA release is increased in the PFC, which would further facilitate PFC activity. Given our observation that the PFC will attenuate HPC activity, this would lead to an attenuation of HPC drive on the striatum which, in turn, will lead to a decrease in mesolimbic DA release. With a fall in mesolimbic DA levels, the PFC input to the striatum will be further facilitated due to the decreased stimulation of presynaptic D2 receptors on the PFC afferents. (C) In contrast, when PFC activity is abnormally attenuated, as may occur in schizophrenia, there would be a reduction in mesocortical DA release as well as a decreased PFC-mediated inhibition of HPC activity. As a consequence, there would be an increase in HPC drive of striatal neuron activity and mesolimbic DA release. The increase in striatal DA D1 and D2 receptor stimulation would then cause a further attenuation of PFC input and facilitation of HPC input to the striatum. Administration of a DA antagonist would then serve to restore the HPC/PFC balance. (+), (–), and (NC) denotes increase, decrease, and no change, respectively.

Shimamura, 2000; Zironi *et al.*, 2001), when there is a higher degree of PFC activity in relation to the HPC, DA release in the VS could be suppressed, whereas higher activity within the HPC would lead to augmented DA release in the VS. Therefore, in schizophrenia, one would postulate that attenuated PFC function

could result in augmented HPC activity, which in turn would cause exaggerated DA release in the VS and further shift toward limbic predominance over PFC control (Fig. 4; Goto and Grace, 2005b; Meyer-Lindenberg *et al.*, 2002, 2005).

Since the discovery that schizophrenia symptoms respond to treatment by D2 antagonists (Carlsson, 1974), the therapeutic approach to treating schizophrenia by antipsychotic drugs has targeted brain DA systems. Nevertheless, examination into a biological basis for this purported hyper-DA state via testing for alterations in D2 receptors has not produced consistent results. Postmortem tissue and imaging studies reporting increased D2 density in schizophrenia have been controversial, with many studies reporting no difference (Farde *et al.*, 1987; Nordstrom *et al.*, 1995). In contrast, given that one of the core deficits in schizophrenia is cognitive dysfunctions that have been associated with PFC activity (Weinberger *et al.*, 1994), studies have focused on alterations of D1 receptors in the PFC of schizophrenia patients. Indeed, although the results are still incomplete, alterations, that is increases (Okubo *et al.*, 1997) or decreases (Abi-Dargham *et al.*, 2002), of D1 receptors in the PFC of schizophrenia patients have been reported. Given that D1 receptor stimulation facilitates calcium influx via NMDA channels (Greengard *et al.*, 1999; Tseng and O'Donnell, 2004), alterations in D1 receptors are also consistent with the hypo-NMDA function theory of schizophrenia pathophysiology (Coyle *et al.*, 2003; Goff and Coyle, 2001; Jentsch and Roth, 1999). This model is based on observations that NMDA antagonists can lead to schizophrenia-like symptoms in normal patients, and moreover can precipitate a recurrence of symptoms in schizophrenia patients that is indistinguishable from that of a relapse (Coyle *et al.*, 2003; Goff and Coyle, 2001; Jentsch and Roth, 1999). Since it is known that DA stimulation of D1 receptors in the PFC exhibits an inverted U-shaped relationship, with optimal DA release required for mediating effective cognitive functions (Lidow *et al.*, 1998; Robbins, 2005), increases or decreases in D1 receptors would shift the relationship between optimal DA release and over- or understimulation of D1 receptors and their impact on PFC function (Callicott *et al.*, 2003; Goto *et al.*, 2004; Manoach, 2003).

The exact mechanism by which D2 antagonism achieves therapeutic efficacy in schizophrenia is still unclear. However, a number of possibilities have been suggested. First, D2 antagonists increase glutamate release from the PFC afferents into the striatum (Brady and O'Donnell, 2004; Goto and Grace, 2005b; O'Donnell and Grace, 1994), facilitating corticostriatal information processing. Alternately, D2 antagonism could affect D2 receptors located within the PFC (Seamans *et al.*, 2001b; Tseng and O'Donnell, 2004; Wang and Goldman-Rakic, 2004; Wang *et al.*, 2004), which may facilitate PFC activity. These two possibilities predict that D2 antagonists should have immediate effects on schizophrenia symptoms. Although studies have suggested that antipsychotic drugs may have an immediate effect on schizophrenia symptoms (Ngan *et al.*, 2002), these results are confounded by the fact that the antipsychotic drugs were tested in patients that

had been withdrawn from treatment. It is known from animal models that prior antipsychotic drug treatment will sensitize the DA system to subsequent administration, dramatically shortening the time required to achieve DA neuron inactivation (Moore *et al.*, 1998). In contrast, most clinical trials of drug-naive patients show a delayed onset of therapeutic actions (Hamill and Fontana, 1975; Johnstone *et al.*, 1978), suggesting other possible mechanisms may be involved. One mechanism that has been consistent with both the pharmacology and time course of antipsychotic drug action is the induction of depolarization block of DA neurons by repeated antipsychotic drug treatment (Grace *et al.*, 1997). It has been shown that repeated administration of D2 antagonists for 3 weeks induces substantial membrane potential depolarization of DA neurons, leading to a cessation of spike discharge (Grace and Bunney, 1986). As a consequence, these DA neurons become unable to evoke spike firing secondary to an overdepolarized membrane potential state. Therefore, unlike the blockade of postsynaptic receptors produced by acute administration of these drugs (that could be overcome by compensatory changes), an abnormal excitatory drive of DA neurons would be incapable of increasing DA release. Therefore, depolarization block of DA neurons could reduce the amount of DA release in the striatum produced in an event-related manner.

VIII. Conclusions

One thing that is clear from the above review is that the DA system exerts complex, multifaceted actions within several interrelated systems of the mammalian brain. It has a role in motor function, motivation and reward, attention, and learning and memory. Therefore, the widespread but anatomically discrete projections of the DA system are positioned to coordinate functions that can have a major impact on cognition and goal-directed behavior. While such diverse functions illuminate the many-faceted disruptions that can occur within this system to lead to a variety of psychiatric disturbances, it also highlights the difficulty in specifically targeting therapeutic agents to single DA systems. A better understanding of the factors that control the DA system, and how they may differentially affect specific DA circuits, may provide the type of insight required to effectively target therapeutic agents.

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GLUTAMATE AND SCHIZOPHRENIA: PHENCYCLIDINE, *N*-METHYL-D-ASPARTATE RECEPTORS, AND DOPAMINE–GLUTAMATE INTERACTIONS

Daniel C. Javitt

Program in Cognitive Neuroscience and Schizophrenia, Nathan Kline Institute for Psychiatric Research, New York University School of Medicine, Orangeburg, New York 10962, USA

Schizophrenia is a serious mental disorder that affects up to 1% of the population worldwide. As of yet, neurochemical mechanisms underlying schizophrenia remain unknown. To date, the most widely considered neurochemical hypothesis of schizophrenia is the dopamine hypothesis, which postulates that symptoms of schizophrenia may result from excess dopaminergic neurotransmission particularly in striatal brain regions, along with dopaminergic deficits in prefrontal brain regions. Alternative neurochemical models of schizophrenia, however, have been proposed

INTERNATIONAL REVIEW OF NEUROBIOLOGY, VOL. 78
DOI: 10.1016/S0074-7742(06)78003-5

0074-7742/07 $35.00

involving glutamatergic mechanisms in general and *N*-methyl-D-aspartate (NMDA) receptors in particular. A potential role for glutamatergic mechanisms in schizophrenia was first proposed ~15 years ago based on the observation that the psychotomimetic agents phencyclidine (PCP) and ketamine induce psychotic symptoms and neurocognitive disturbances similar to those of schizophrenia by blocking neurotransmission at NMDA-type glutamate receptors. Since that time, significant additional evidence has accumulated supporting a role for NMDA hypofunction in the pathophysiology of schizophrenia. Clinical challenge studies with PCP and ketamine have confirmed the close resemblance between NMDA antagonist-induced symptoms and neurocognitive deficits and those observed in schizophrenia, and suggest that NMDA dysfunction may lead to secondary dopaminergic dysregulation in striatal and prefrontal brain regions. As compared to dopaminergic agents, NMDA antagonists induce negative and cognitive symptoms of schizophrenia, as well as positive symptoms. Treatment studies with NMDA modulators, such as glycine, D-serine, and glycine transport inhibitors (GTIs), have yielded encouraging findings, although results remain controversial. Finally, genetic linkage and *in vivo* neurochemical studies in schizophrenia highlight potential etiological mechanisms giving rise to glutamatergic/NMDA dysfunction in schizophrenia.

I. Introduction

Schizophrenia is a serious mental disorder that affects up to 1% of the population worldwide, and is one of the leading causes of chronic disability. The first effective treatments for schizophrenia were discovered fortuitously in the late 1950s, and subsequently shown to mediate their effects at dopamine D2 receptors. Since that time, dopamine has been the primary neurotransmitter implicated in schizophrenia, and the majority of neurochemical studies of schizophrenia continue to focus on dopaminergic mechanisms (Carlsson, 1988).

Neurochemical models of schizophrenia based on dopaminergic theories have had substantial heuristic value in explaining key symptoms of schizophrenia, in particular, positive symptoms, and in guiding treatment considerations. For example, all antipsychotics are effective at doses that occupy ~80% of brain D2 receptors (Kapur and Remington, 2001). Further, individuals with schizophrenia do show enhanced striatal dopamine release to amphetamine challenge at least during the acute stage of illness (Laruelle, 1998). Nevertheless, significant limitations with regard to the dopamine hypothesis remain. First, no intrinsic deficits have been observed within the dopamine system to account for the presumed hyperdopaminergia associated with schizophrenia. Second, reconceptualizations

of the dopamine hypothesis propose that subcortical hyperdopaminergia may coexist with cortical hypodopaminergia (Davis *et al.*, 1991), although mechanisms underlying the differential cortical and subcortical abnormalities remain to be determined. Finally, dopaminergic dysfunction, in general, accounts poorly for symptom classes in schizophrenia other than positive symptoms, and for the pattern of neurocognitive dysfunction associated with schizophrenia. Thus, alternative conceptual models of schizophrenia are required.

An alternative to the dopamine model was first proposed in the early 1990s, based on the observation that phencyclidine (PCP) and similarly acting psychotomimetic compounds induced their unique behavioral effects by blocking neurotransmission at *N*-methyl-D-aspartate (NMDA)-type glutamate receptors (Javitt, 1987; Javitt and Zukin, 1991). The ability of these compounds to transiently reproduce key symptoms of schizophrenia by blocking NMDA receptors led to the concept that symptoms in schizophrenia may reflect underlying dysfunction or dysregulation of NMDA receptor-mediated neurotransmission.

Over the past 15 years, convergent evidence has accumulated to support a primary role for glutamatergic dysfunction in the pathophysiology of schizophrenia (Abi-Saab *et al.*, 1998; Coyle, 1996; Olney *et al.*, 1999; Tamminga *et al.*, 1995). In particular, studies have documented a close congruence between symptomatic and neurocognitive effects induced by NMDA antagonists such as PCP and the related drug ketamine, and the pattern observed in schizophrenia. Further, both genetic and neurochemical studies have begun to identify pathogenetic events that may impact on glutamatergic neurotransmission, and provide plausible bases for underlying NMDA dysfunction. Finally, evidence from both animal and human studies suggest that the hyperdopaminergia associated with schizophrenia may, in fact, result from underlying dysfunction of NMDA-related neuromodulatory feedback mechanisms. Overall, these findings suggest new etiological and psychotherapeutic conceptualizations of schizophrenia.

II. Glutamatergic Physiology

Glutamate is the primary excitatory neurotransmitter in brain, accounting for roughly 60% of neurons and 40% of synapses. Virtually all cortical pyramidal neurons use glutamate as their primary excitatory neurotransmitter. Glutamate is synthesized in brain from glutamine, which is transported across the blood–brain barrier with high affinity and present at high concentration in extracellular brain fluid and cerebrospinal fluid. Following release, glutamate is reabsorbed by both neuronal and glial glutamate transporters via an energy-dependent transport process. Much of the brain energy demand relates to glutamate homeostasis.

A. Glutamate–Dopamine Comparisons

To date, dopaminergic models of schizophrenia have emphasized primary dysfunction of circumscribed brain pathways, such as the mesocortical and mesolimbic dopamine systems, as being the primary pathophysiological events in schizophrenia, with other types of deficits deriving secondarily from such disturbances. Given the widespread distribution of glutamate in the brain, however, glutamatergic models start from a different conceptual framework.

In glutamatergic models, it is presumed that similar functional disturbances are present throughout cortex and that such deficits may even involve subcortical glutamatergic pathways. Thus, glutamatergic models differ from dopaminergic models in that they predict widespread, rather than circumscribed, patterns of cortical dysfunction in schizophrenia. Nevertheless, not all glutamate receptors appear to be involved equally. In particular, clinical features of schizophrenia are most consistent with circumscribed dysfunction NMDA receptors, and new treatment approaches for schizophrenia are increasingly targeting these receptors. NMDA receptors, however, work in association with other glutamatergic and nonglutamatergic receptors. Such interactions may play a critical role as well in both etiopathology and treatment of schizophrenia.

B. Glutamate Receptors

Receptors for glutamate are divided into two broad families. Ionotropic receptors are differentiated based on sensitivity to the synthetic glutamate derivatives NMDA, AMPA, and kainate. Metabotropic receptors, which are G-protein coupled and mediate longer-term neuromodulatory effects of glutamate, are divided into groups on the basis of effector coupling and ligand sensitivity. Despite the differential sensitivity of these receptors to specific synthetic ligands, the endogenous neurotransmitter for all receptors is glutamate, and, to a lesser extent, the closely related amino acid aspartate.

C. NMDA Receptors

NMDA receptors (Fig. 1) are the most complex of the ionotropic receptors. In addition to the recognition site for glutamate, NMDA receptors contain an allosteric modulatory site that binds the endogenous brain amino acids glycine and D-serine. This glycine-binding site, like the benzodiazepine site of the $GABA_A$ receptor, regulates channel open time and desensitization rate in the presence of agonist (glutamate), but does not, of itself, induce channel opening. Like the benzodiazepine site, therefore, this may be an ideal target for drug development.

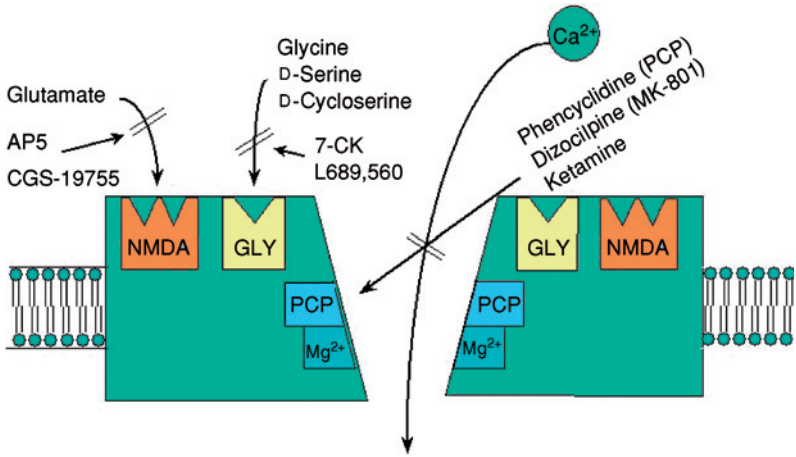

FIG. 1. Schematic model of the NMDA receptor complex.

Both glycine and D-serine are present in high concentration in brain. However, NMDA receptors appear to be protected from circulating glycine/D-serine level by the presence of amino acid transporters that are colocalized with NMDA receptors. Glycine type I (GLYT1) transporters may play a key role, although other small neutral amino acid transporters (SNATs) may also contribute (Javitt *et al.*, 2005a). As with the glycine site itself, these transporters have become a prominent target for drug development.

In addition to the glycine modulatory site, the NMDA receptor complex contains regulatory sites that are sensitive to polyamines, Zn^{2+}, protons, and redox agents such as glutathione. The multiple influences that converge on NMDA receptors speak to the critical role played by these receptors in a multitude of brain processes and suggest additional potential sites for therapeutic intervention.

NMDA receptors are blocked in a voltage sensitive fashion by Mg^{2+}, which binds to a site within the NMDA ion channel. As a result, NMDA receptors are uniquely voltage- as well as ligand (glutamate)-sensitive. This property permits NMDA receptors to play a unique role in the regulation of connection strength between neurons through a process known as long-term potentiation (LTP), and to act in a "Hebbian" fashion to integrate input from multiple independent pathways. In addition, because NMDA receptors can be turned "on" or "off" simply by varying the membrane voltage, they serve as a key elements in circuits related to attention, gating, and feedback regulation.

NMDA receptors are composed of multiple subunits, including at least one NR1 subunit and one or more modulatory subunits from the NR2

(NR2A–NR2D) and/or NR3 (NR3A, NR3B) families. These subunits significantly alter the functional properties of native NMDA receptors, including their voltage sensitivity, peak conductance, and degree to which they are influenced by the endogenous modulators glycine and D-serine. Interestingly, while the modulatory agents glycine and D-serine have similar, excitatory effects on NMDA receptors containing NR2 subunits, they have opposite effects on receptors containing NR3 subunits, with glycine serving to activate NR3-containing receptors and D-serine to inhibit them (Chatterton *et al.*, 2002).

NMDA receptors are blocked in a noncompetitive fashion by PCP, ketamine, and other agents such as dizocilpine (MK-801), which bind to a site (PCP receptor) located within the ion channel formed by the NMDA complex. The ability of NMDA antagonists to induce schizophrenia-like psychotic symptoms is among the strongest evidence to date linking glutamatergic dysfunction to the pathophysiology of schizophrenia.

D. AMPA/Kainate Receptors

AMPA/kainate receptors are a second class of ionotropic receptors for the neurotransmitter glutamate. AMPA receptors are composed of combinations of GluR1–4 subunits, while kainate receptors are composed of GluR5–7 and KA1 and KA2 subunits. Both receptor types interact closely with NMDA receptors, although at present the role of AMPA receptors is better understood, especially with regard to LTP. LTP is a fundamental process in the brain by which the strength of connections between neurons is modulated. It is thus the basis for much of learning, memory, and synaptic plasticity. Modulation of connection strength between neurons has long been known to be initiated by Ca^{2+} flux through open, unblocked NMDA channels. More recently, the interplay among glutamate receptors that permits such alterations in connections strength has also been evaluated.

Mature AMPA receptors containing the GluR2 subunit are Ca^{2+} impermeant (Tanaka *et al.*, 2000), and thus do not directly trigger LTP. Nevertheless, AMPA receptors provide the primary depolarization necessary to unblock NMDA receptors and to permit calcium entry into the cell. Ca^{2+} entry through unblocked NMDA receptors, in turn, triggers AMPA insertion into the postsynaptic density and synaptic strengthening. Thus, activity at AMPA and NMDA receptors is needed for coordinated glutamatergic neurotransmission.

The inverse of the synergistic relationship is that dysfunction of either AMPA or NMDA receptors may lead to the phenomenon of the silent synapse. AMPA receptors are continuously recycled, leading to gradual synaptic weakening. If AMPA density falls below a critical threshold, levels of depolarization are insufficient to unblock NMDA channels, preventing postsynaptic depolarization

or Ca^{2+} influx. The lack of Ca^{2+} influx precludes subsequent AMPA receptor insertion into the postsynaptic membrane. Thus, such synapses, despite containing histologically identifiable NMDA receptors, are functionally silent and cannot be recovered by electrical stimulation alone (Isaac *et al.*, 1999). To the extent that it occurs in schizophrenia, the silencing of synapses may limit the degree of recovery to be expected even if normal glutamatergic functioning could be restored.

E. Metabotropic Receptors

As opposed to ionotropic receptors, which are linked directly to ion channels, metabotropic receptors are linked to second messenger systems and affect neuronal metabolism. A particular role of glutamatergic metabotropic receptors is regulation of presynaptic glutamate release and postsynaptic sensitivity. Metabotropic receptors are divided into three groups based on functional activity. Group I (types 1 and 4) receptors function predominantly to potentiate both presynaptic glutamate release and postsynaptic NMDA neurotransmission. In contrast, Group II (types 2 and 3) and Group III (types 5–7) receptors serve to limit glutamate release, particularly during conditions of glutamate spillover from the synaptic cleft. Thus, Group I agonists would be expected to stimulate neurotransmission mediated by ionotropic glutamate receptors, whereas agonists for Group II/III receptors would be expected to have opposite effects.

III. Glutamatergic Models of Schizophrenia

The strongest evidence linking glutamate in general and NMDA receptors in particular to the pathophysiology comes from studies of PCP and other "dissociative anesthetics" such as ketamine. Although the overall similarity between NMDA antagonist-induced psychosis has been appreciated since the early 1960s, studies continue to refine the relationships between the two clinical states.

Symptoms of schizophrenia are currently divided into at least three independent factors, labeled positive, negative, and cognitive or disorganized symptoms, respectively on the basis of rating scales such as the Psychiatric Rating Scale (BPRS) (Overall and Gorham, 1961) or the Positive and Negative Symptom Scale (PANSS) (Kay *et al.*, 1987). The positive factor consists of items such as hallucinations, paranoia, and agitation, while the negative factor consists of items such as apathy, motor retardation, and emotional withdrawal. The "cognitive" or "disorganized" factor, while more variable across studies, tends to include items such as conceptual disorganization, difficulties in abstract thinking, mannerisms,

and poor attention. Although the absolute level of symptoms varies across subjects, factor scores tend to be correlated both cross-sectionally and longitudinally. Thus, all patients with schizophrenia show at least some degree of negative and cognitive symptoms, along with positive.

In addition to symptoms, patients with schizophrenia show a pattern of neurocognitive dysfunction that represents a core feature of the disorder. Neurocognitive deficits are present at first episode and cannot be attributed to effects of medication. At present, it appears that patients who subsequently develop schizophrenia may have some level of neurocognitive dysfunction even during childhood but that further neurocognitive decline occurs in the years immediately preceding psychotic decompensation (Lieberman *et al.*, 2001). As with symptoms, the underlying neurochemical basis of neurocognitive dysfunction in schizophrenia has not been determined.

Finally, over recent years, specific neurochemical features of schizophrenia, including subcortical hyperdopaminergia and white matter degeneration, have been defined based on *in vivo* imaging and postmortem analysis. As the schizophrenia phenotype has been increasingly well characterized, it has been possible to assess with increasing precision the degree to which NMDA dysfunction may account for specific symptoms and features of schizophrenia.

A. Symptom Patterns Following NMDA Antagonist Administration

In initial studies with PCP and ketamine in the early 1960s, it was noted that both agents produced what would now be considered positive, negative, and cognitive symptoms of schizophrenia (Javitt and Zukin, 1991; Luby *et al.*, 1962). At the time, however, no formal rating scales were used. In subsequent studies using such rating scales, however, significant increases are observed not only in positive symptoms but also in negative symptoms and disorganization (Krystal *et al.*, 1994; Lahti *et al.*, 2001; Malhotra *et al.*, 1996; Newcomer *et al.*, 1999). Levels of symptoms during acute ketamine challenge tend to show a similar pattern across factors as they do in schizophrenia. When patients with schizophrenia are exposed to ketamine, they also show increases in positive symptoms, as well as negative symptoms (Lahti *et al.*, 2001; Malhotra *et al.*, 1996). Ketamine induced symptoms respond poorly to conventional antipsychotics, but may be reversed particularly by clozapine treatment (Malhotra *et al.*, 1997a).

Despite the overall similarity between ketamine-induced symptoms and those of schizophrenia, there are some potentially informative differences. In particular, patients with schizophrenia often report hearing voices, while during ketamine administration such symptoms are rare. In addition, visual perceptual distortions are common during ketamine infusion but rare in established cases of schizophrenia. Nevertheless, the pattern of auditory and visual disturbances seen during

ketamine administration does resemble the pattern observed early in the course in schizophrenia (McGhie and Chapman, 1961) where both auditory and visual perceptual disturbances are common, and auditory hallucinations have not yet crystallized to the point of being identifiable as speech. Thus, acute ketamine challenge may be viewed as a model of acute incipient schizophrenia, rather than later, more chronic, phases. In patients with established schizophrenia, increases in hallucinatory activity are observed during ketamine challenge (Lahti *et al.*, 2001; Malhotra *et al.*, 1997b). Further, in primates, hallucinatory behavior is not observed during acute PCP treatment but does emerge during chronic administration (Linn *et al.*, 1999). In humans, for obvious ethical reasons, effects of chronic ketamine or PCP treatment are not well characterized.

Although symptomatic effects of ketamine and amphetamine have been extensively investigated in separate investigations, relatively few studies have tested both sets of compounds within the same volunteer subjects. A study, however, confirms results of prior independent investigations (Krystal *et al.*, 2005). In that study, effects of ketamine and amphetamine were assessed both individually and in combination. Consistent with prior studies, ketamine induced both positive and negative symptoms in approximately equal proportions—that is, similar to the type of levels seen in schizophrenia—whereas amphetamine induced only positive symptoms with no significant effect on negative symptoms.

The specific pattern of symptoms induced by amphetamine and ketamine differed as well. Thus, amphetamine induced significant increases in grandiosity, but not delusions, whereas ketamine induced significant increases in delusions but not grandiosity. Ketamine also produced greater degrees of hallucinatory behavior than did amphetamine. Additive effects between amphetamine and ketamine were seen only in the case of hallucinations, suggesting that the circuitry underlying hallucinations may have unique sensitivity to both glutamatergic and dopaminergic dysfunctions.

Similarly, although both amphetamine and ketamine produced increases in the cognitive factor score, the pattern of symptoms induced by the two compounds differed significantly. While amphetamine increased scoring primarily on one element of the cognitive symptom factor, conceptual disorganization, it had no effect on other cognitive symptoms. In contrast, ketamine significantly increased schizophrenia-like deficits not only in conceptual disorganization but also in difficulties in abstract thinking, mannerisms, and poor attention. Although some additivity between amphetamine and ketamine effects was observed, the degree of interaction did not reach statistical significance.

Overall, these findings continue to support the close similarity between NMDA antagonist-induced symptoms and those observed in schizophrenia. In contrast, patterns of symptoms induced by dopaminergic agonists, such as amphetamine, differ markedly from those seen in schizophrenia. Although there is a tendency to attribute positive symptoms in schizophrenia to dopaminergic hyperactivity and

negative symptoms to disturbances in other brain pathways, ketamine challenge studies to date suggest that NMDA dysfunction, by itself, would be sufficient to account for pathogenesis of all three symptom dimensions (positive, negative, cognitive) most associated with schizophrenia.

B. Cognitive Deficits Following NMDA Antagonist Treatment

In addition to symptoms, schizophrenia is associated with a pattern of neurocognitive dysfunction that is highly characteristic of the disorder (Bilder *et al.*, 1991; Gold *et al.*, 1999). Based in part on the influence of dopaminergic models of schizophrenia, a great number of cognitive studies in schizophrenia have focused on dysfunction of specific brain regions such as prefrontal dysfunction. However, neurocognitive deficits in schizophrenia are in no ways limited to prefrontal function. In studies that have utilized comprehensive neuropsychological batteries, similar levels of deficit have been observed across widespread neurocognitive domains, with preferential deficits, if any, observed in learning and declarative memory formation (Bilder *et al.*, 2000; Dickinson *et al.*, 2006; Keefe *et al.*, 2006; Saykin *et al.*, 1991).

A challenge in the neuropsychological literature has been to define a single brain abnormality that could account for the complex array of neuropsychological deficits seen in schizophrenia. Thus, while patients have many deficits attributable to prefrontal dysfunction in some tests (MacDonald *et al.*, 2005), on other prefrontal tests the pattern of deficit is incompatible with local structural disturbance (Shurman *et al.*, 2005). Further, patients show deficits even in simple visual (Butler *et al.*, 2005) and auditory (Strous *et al.*, 1995; Wexler *et al.*, 1998) sensory processing, with pattern of deficit indicating dysfunction within primary sensory regions (Rabinowicz *et al.*, 2000). Thus, no single structural deficit within cortex can give rise to the complex pattern of neurocognitive dysfunction seen in schizophrenia.

Glutamatergic models provide a potential alternative framework from which to view the pattern of neuropsychological dysfunction associated with schizophrenia. Although glutamatergic systems are widespread, within each brain region NMDA receptors participate in only a subset of processes. For example, in hippocampus and cortex, NMDA receptor activation is required for the initiation, but not maintenance of LTP (Miyamoto, 2006). The observation that patients with schizophrenia (as opposed to those with the amnestic syndrome) show deficits in memory formation (Hartvig *et al.*, 1995; Krystal *et al.*, 2005; Morgan *et al.*, 2004a; Newcomer *et al.*, 1999; Parwani *et al.*, 2005; Radant *et al.*, 1998; Rowland *et al.*, 2005), but not retention, is thus consistent with an NMDA pattern of dysfunction within hippocampal regions, rather than structural damage to the hippocampus itself.

To date, a substantial literature has accumulated comparing effects of NMDA antagonists to those observed in schizophrenia, using paradigms sensitive to both sensory and cognitive aspects of information processing dysfunction in schizophrenia. Deficits have been observed across widespread neuropsychological domains, including working memory (Honey *et al.*, 2003; Morgan *et al.*, 2004a), response inhibition (Morgan *et al.*, 2004b), procedural memory (Morgan *et al.*, 2004a), and executive processing (Krystal *et al.*, 1994; Umbricht *et al.*, 2000). Further, deficits are observed in sensory processing as well. For example, ketamine administration inhibits generation of mismatch negativity (MMN), an event-related potential (ERP) component that reflects impaired information processing at the level of auditory cortex (Umbricht *et al.*, 2000), and alters proprioceptive performance (Oye *et al.*, 1992). Similarly, administration of NMDA antagonists in rodents produces a pattern of visual ERP deficit similar to that observed in schizophrenia (Butler *et al.*, 2005). Ketamine infusion also reproduces both the severity and type of thought disorder seen in schizophrenia with both, for example, being associated with high levels of poverty of speech, circumstantiality and loss of goal, and relatively low levels of distractive or stilted speech or paraphasias (Adler *et al.*, 1999). Thus, reduction in NMDA functioning within brain could serve as a single unifying feature to account for the otherwise complex pattern of deficit observed in the disorder.

As opposed to ketamine, administration of dopaminergic agonists such as amphetamine does not reproduce the pattern of deficit observed in schizophrenia. Further, several recent studies have assessed the ability of amphetamine to improve neurocognitive performance in schizophrenia, on tasks such as the Stroop test. In this test, patients showed a characteristic pattern of deficit characterized by increased facilitation of response by stimulus congruence. Although amphetamine improved overall performance in this task in both normal and schizophrenia subjects, it nonetheless failed to reverse the specific pattern of neurocognitive dysfunction associated with schizophrenia (Barch and Carter, 2005). On the basis of these findings, dysfunction of dopaminergic systems appears neither necessary nor sufficient to account for the overall pattern of neuropsychological disturbance in schizophrenia, although interactions between dopaminergic and glutamatergic systems may occur.

C. *In Vivo* Findings in Schizophrenia Based on Dopamine Receptor Occupancy

Along with neurocognitive studies, which provide insights into patterns of cortical dysfunction in schizophrenia, positron emission (PET) and single photon emission (SPECT) *in vivo* tomographic studies provide insights into patterns of dopaminergic dysfunction in schizophrenia. In such studies, D2 agonists are tagged

with appropriate radionuclides (e.g., [14C], [123I]) and pattern of displacement is evaluated following administration of a dopaminergic agent. Increased synaptic dopamine levels are associated with reduced binding potential of D2 ligands. Striatal and cortical dopaminergic circuits are known to be under regulatory control by glutamatergic systems (Carlsson, 2006; Javitt and Zukin, 1991; Kulagina *et al.*, 2001). Such studies permit *in vivo* assessment of dopamine–glutamate interactions.

Both amphetamine (Breier *et al.*, 1998; Laruelle *et al.*, 1995) and ketamine (Breier *et al.*, 1998; Smith *et al.*, 1998; Vollenweider *et al.*, 2000) decrease striatal binding potential of D2 ligands following acute administration in humans, suggesting that both increase striatal dopamine levels. Thus, the presumed subcortical hyperdopaminergia of schizophrenia could result from either underlying dopaminergic hyperactivity or NMDA hypoactivity. Dissociative effects of ketamine, however, have been observed even under administration conditions that do not acutely affect striatal dopamine levels (Kegeles *et al.*, 2002), suggesting that psychotomimetic effects of ketamine cannot be attributed to alterations in dopaminergic function alone.

Objective assessment of dopamine function has been operationalized in schizophrenia using amphetamine challenge. Across cohorts, patients with acute schizophrenia show enhanced striatal dopamine release to amphetamine challenge, consistent with presumed dysregulation of subcortical dopamine circuits (Laruelle *et al.*, 1999). Levels of dopamine increase, moreover, correlate with severity of amphetamine-induced positive symptoms (Laruelle *et al.*, 1996). Deficits similar to those observed in schizophrenia are observed as well in normal volunteers undergoing ketamine infusion (Kegeles *et al.*, 2000), and in rodents treated acutely (Miller and Abercrombie, 1996) or subchronically (Balla *et al.*, 2001) with NMDA receptor antagonists. In nonhuman primates, the metabotropic agonist LY354740 also potentiates amphetamine-induced dopamine release (van Berckel *et al.*, 2006), by inhibiting presynaptic glutamate release, further suggesting that deficits in glutamatergic functioning may underlie dopaminergic hyperreactivity in schizophrenia.

Although initial *in vivo* studies in schizophrenia focused primarily on striatal functioning, dopamine receptor occupancy studies have now been performed in cortex as well. For example, Aalto *et al.* (2005) have demonstrated that acute ketamine administration increases dopamine release in cortex, as well as in striatum, and that the increase in release correlates with severity of ketamine-induced psychotic symptoms. Narendran *et al.* (2005) have demonstrated increases in D1 receptor binding in chronic ketamine abusers, suggesting also that ketamine may modulate prefrontal dopaminergic neurotransmission. Similar effects are observed in primates, in which chronic treatment with NMDA antagonists reduced tonic dopamine levels and D1 receptor upregulation, along with deficits in working memory (Tsukada *et al.*, 2005). In rodents, subchronic

treatment with NMDA antagonists induces enhanced amphetamine-induced dopamine release in prefrontal cortex (Balla *et al.*, 2003), suggesting that schizophrenia may be associated with reduced tonic dopamine levels as well with prefrontal hyperdopaminergia.

At present, radioligand binding studies of D1 receptor binding in schizophrenia have yielded conflicting results, with individual studies showing decreases (Okubo *et al.*, 1997), no change (Karlsson *et al.*, 2002) and increases (Abi-Dargham, 2003) in schizophrenia subjects, with discrepancy between studies most likely reflecting differences in patient and receptor ligand binding characteristics.

D. Postmortem Findings

Postmortem studies provide a final source of information that can be used to evaluate potential explanatory value of glutamatergic models. Schizophrenia is associated with complex patterns of alterations in protein and gene expression that cannot be easily explained based on dopaminergic models alone. For example, robust and reproducible deficits in parvalbumin and GAD67 expression are observed in postmortem hippocampus and prefrontal cortex in schizophrenia subjects (Reynolds *et al.*, 2004; Torrey *et al.*, 2005), although underlying mechanisms are unknown. NMDA receptors regulate both parvalbumin and GAD67 expression in cultured GABAergic interneurons, with ketamine leading to reduced expression of both agents (Kinney *et al.*, 2006). Similarly, subchronic treatment with PCP in rodents leads to downregulation of parvalbumin expression *in vivo* (Abdul-Monim *et al.*, 2006; Reynolds *et al.*, 2004). Other deficits, such as altered levels of *N*-acetylaspartate (NAA), *N*-acetylaspartylglutamate (NAAG) (Tsai *et al.*, 1995), and carboxypeptidase (Ghose *et al.*, 2004) may also be reproduced by subchronic PCP administration (Flores and Coyle, 2003; Reynolds *et al.*, 2005). Effects of NMDA antagonists have only been tested on a minority of postmortem markers of schizophrenia, and effects of antipsychotic agents in postmortem studies cannot always be excluded. Nevertheless, studies performed to date suggest that glutamatergic models may be able to explain postmortem as well as *in vivo* findings in schizophrenia.

IV. Clinical Studies with NMDA Agonists

To date, all approved agents for treatment of schizophrenia function by blocking neurotransmission at D2-type dopamine receptors. Given the hypothesis that NMDA dysfunction may underlie both clinical symptoms and neurocognitive dysfunction associated with schizophrenia, a critical issue is whether glutamate

TABLE I

Summary of Clinical Findings with the Full *N*-Methyl-d-aspartate Receptor Glycine-Site Agonists Glycine and d-Serine and the Partial Agonist d-Cycloserine in Combination with Typical, Atypical, or Mixed Antipsychotics in Schizophrenia

				Negative		Cognitive		Positive	
Study	Agonist[a]	Antipsychotic[b]	*N*	Change (%)	*p*	Change (%)	*p*	Change (%)	*p*
Heresco-Levy *et al.* (1999)	GLY	Mixed	22[c]	−39	<0.001	−24	0.01	−20	NS
Javitt *et al.* (2001)	GLY	Mixed	12[c]	−34	<0.05	−11.9	0.1	−11	0.08
Heresco-Levy *et al.* (2004)	GLY	Olz/Risp	17[c]	−23	<0.0001	−9.2	0.02	−11.4	0.006
Evins *et al.* (2000)	GLY	Clozapine	27	−4	NS	–	–	−7	NS
Tsai *et al.* (1998)	DSER	Mixed	29	−20	<0.001	−17.7	0.004	−21.9	0.004
Heresco-Levy *et al.* (2005)	DSER	Olz/Risp	39[c]	−16	<0.001	−11.7	0.001	−13	0.001
Tsai *et al.* (1999)	DSER	Clozapine	20	−2.5	NS	−0.8	NS	−3.6	NS
Tsai *et al.* (2005)	d-Alanine	Mixed	32	−17	<.0001	−13	<.0001	−13	.0002
Goff *et al.* (1999b)	DCS	Conventional	47	−23	<.02[b]	–	–	–	NS
Heresco-Levy *et al.* (2002)	DCS	Mixed	21[c]	−14	<.05	–	–	–	NS
Goff *et al.* (1999a)	DCS	Clozapine	17[c]	+13[d]	<.005	–	–	–	NS
Tsai *et al.* (2004a)	Sarcosine	Mixed	38	−14	<.0001	−17	<.0001	−13	<.0001
Lane *et al.* (2006)	Sarcosine	Clozapine	20	−2.1	NS	−9.1	NS	−6.6	NS

agonists can ameliorate persistent symptoms of schizophrenia. The glutamate-binding site of the NMDA and AMPA receptors cannot easily be targeted because of fear of seizures, excitotoxicity, and other evidence of cortical hyperexcitability. Further, these sites are not designed to be tonically occupied by agonist but instead must be physically activated analogously to other ionotropic receptors (e.g., nicotinic cholinergic, $GABA_A$). Instead, current strategies have focused on mechanisms for increasing efficiency of both NMDA- and AMPA-mediated neurotransmissions without altering levels of glutamate itself. For NMDA receptors, studies have targeted primarily the glycine modulatory site, in part because of the availability of naturally occurring compounds that permitted early stage clinical investigations. A "second generation" approach has been the use of glycine (GLYT1) transport inhibitors, which lead to increases in brain glycine levels by blocking its removal from the synaptic space. Finally, early stage trials have been conducted with allosteric AMPA receptor modulators (AMPAkines), as well as metabotropic receptor agonists and antagonists.

A. NMDA Receptor Glycine-Site Agonists

To date, the majority of clinical studies based on the glutamate hypothesis have been conducted with positive allosteric modulators of the NMDA receptor complex. Three separate agents have been used for these studies: glycine and D-serine, which function as full agonists, and D-cycloserine, which functions as a partial agonist. Glycine has been used primarily at doses of ~0.4–0.8 g/kg/day (~30–60 g/day); D-serine, at a dose of 30 mg/kg/day (~2.1 g/day) and D-cycloserine at a dose of 50 mg/day. In addition, one study has been performed with D-alanine, a close structural analogue of D-serine, used at a dose of 100 mg/kg/day (~7 g/day) (Table I). With glycine and D-serine, effectiveness of higher doses has not been explored so that maximal benefit obtainable from glycine-site stimulation is unknown. With D-cycloserine, doses in excess of 100 mg cause symptom exacerbation due to emergent NMDA receptor antagonist effects, producing a narrow therapeutic window (van Berckel *et al.*, 1999).

To date, 11 studies have been performed involving over 250 subjects (Table I). All studies involved patients with persistent negative symptoms while on stable

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[a]GLY, glycine (0.4–0.8 g/kg/day); DSER, D-serine (30 mg/kg/day); DALA, D-alanine (100 mg/kg/day); DCS, D-cycloserine (50 mg/day); SARC, sarcosine (30 mg/kg/day).

[b]OLZ, olanzapine; RISP, risperidone.

[c]Crossover study.

[d]Significant difference with SANS only; PANSS difference not significant, positive value represents significant worsening of symptoms.

–, Not determined; NS, not significant.

dose of antipsychotic medication. NMDA agonists were added as adjunctive medication while patients remained on prior antipsychotic regimen. All studies published to date have demonstrated large effect-size (0.9–2.1 SD units) improvement in negative and cognitive symptoms when these agents are added to typical antipsychotics, or newer atypicals. Percentage improvement in negative symptoms range from 16% to 39% (weighted mean 22.5%) for trials in the range of 6–12 weeks. Whether greater reduction occurs during longer-term treatment, or whether tolerance develops, is currently unknown.

The level of cognitive and positive symptom improvement, across studies, is roughly 15%, suggesting the possibility of adjunctive benefit in combination with antipsychotics. Given the ability of NMDA antagonists to induce positive as well as negative symptoms during acute challenge studies, it is not clear whether the greater effect of NMDA agonists on negative, as compared with positive, symptoms is due to an intrinsic property of the approach, or rather to the fact that positive symptoms are already partially treated by antipsychotic medication. Ultimately, monotherapy or antipsychotic withdrawal studies will be required to address these possibilities.

In some, but not all studies with glycine, the degree of negative symptoms improvement has correlated significantly with baseline glycine levels, suggesting that patients with lowest pretreatment levels respond best to NMDA receptor agonist treatment (Heresco-Levy *et al.*, 1999). With glycine, the critical plasma level for therapeutic response appears to be in the range of 600–1000 μM versus a basal level of ∼200 μM. Similar levels have been observed in animal studies (Javitt *et al.*, 2004). The mean percentage change associated with glycine treatment in these studies was ∼30%, suggesting that if these findings could be replicated results would be clinically meaningful.

Partial agonist effects: As compared with full glycine-site agonists, the partial agonist D-cycloserine has proven less efficacious across clinical sites (Table I), and across studies within clinical site (Heresco-Levy *et al.*, 1998). A meta-analysis of clinical studies performed through 2004 found evidence of significant beneficial effect of full glycine-site agonists across studies, but not of D-cycloserine (Tuominen *et al.*, 2005). Despite its overall lack of efficacy, however, D-cycloserine was reported to increase temporal lobe activation during a word recall task, with effects correlating with degree of reduction in negative symptoms (Yurgelun-Todd *et al.*, 2005). Thus, specific beneficial effects may occur over short-term treatment, although it is postulated that tolerance may occur during longer term trials.

NMDA agonists in combination with clozapine: Relative to effects in combination with typical or newer atypical antipsychotics, glycine-site agonists have proven less effective when combined with clozapine. In double blind, placebo-controlled studies in which glycine (Evins *et al.*, 2000) or D-serine (Tsai *et al.*, 1999) have been added to clozapine, no significant beneficial response has been observed,

while D-cycloserine is reported to lead to worsening of symptoms when used in combination with clozapine (Goff *et al.*, 1996).

D-Cycloserine functions as a glycine-site agonist in the presence of high glycine concentrations, and as an antagonist in the presence of high concentrations (Hood *et al.*, 1989). A parsimonious explanation for the differential effects of NMDA agonists in combination with clozapine versus other antipsychotic agents, therefore, is that clozapine may already increase synaptic glycine levels through as yet unknown mechanisms. Recently, clozapine has recently been shown to block glycine and glutamine transport mediated by SNAT2-like synaptosomal transporters, providing a potential mechanism for both the differential therapeutic effects of clozapine and the differential effects of NMDA receptor modulators in the presence of clozapine versus other antipsychotics (Javitt *et al.*, 2005a). This finding may also account for the reported ability of clozapine to increase serum glutamate levels (Evins *et al.*, 1997) and downregulate central glutamate transport (Melone *et al.*, 2003; Pietraszek *et al.*, 2002).

CONSIST: In addition to published studies, one additional study (CONSIST) has been presented to date only in abstract form (Buchanan *et al.*, 2004). In that study, glycine (60 g/day) and D-cycloserine (50 mg/day) were compared versus placebo as adjunctive medication for persistent negative symptoms, using inclusion/exclusion criteria designed to enrich recruitment for individuals meeting criteria for the deficit syndrome (Carpenter *et al.*, 1988). Study duration was 16 weeks. Patients were on stable doses of antipsychotics other than clozapine. In that study, no significant beneficial effects were observed for either glycine or D-cycloserine, although subgroup analyses showed significant beneficial effects of glycine in inpatients and in patients receiving conventional medications only. No significant beneficial effects on cognition were observed in any group. Overall, larger trials are required, possibly with enriched inpatient populations.

Use of glycine in the schizophrenic prodrome: The majority of studies with NMDA agonists have focused on individuals well advanced in their illness. Recently, however, glycine was used in an open label monotherapy study in 10 individuals showing prodromal signs of schizophrenia. Although the number of subjects was limited, three met early remission criteria, one other showed substantial improvement, and two showed moderate improvement. Across all subjects, large effect size changes were observed across both positive and negative domains. Effects of glycine were more pronounced that those that had been observed in a prior double blind study of olanzapine (Woods *et al.*, 2004). These data, if confirmed, would indicate that NMDA agonists might have a primary role in the earliest stages of schizophrenia psychosis, with potential impact across symptomatic domains.

B. Glycine Transport Inhibitors

Both glycine and D-serine appear to be effective when used in treatment resistant schizophrenia. However, both must be given at gram-level doses in order to significantly elevate CNS levels. An alternative approach to increasing CNS levels is use of glycine transport inhibitors (GTIs), which raise synaptic glycine levels by preventing its removal from the synaptic cleft. Use of GTIs to augment NMDA functioning is analogous to use of selective serotonin reuptake inhibitors (SSRIs) to raise synaptic serotonin levels in depression.

Initial studies were performed using the relatively nonselective glycine transport antagonist, glycyldodecylamide (GDA). This drug was shown to inhibit glycine transport in cortical (Javitt and Frusciante, 1997) or hippocampal (Harsing *et al.*, 2001) synaptosomes, and inhibit amphetamine-induced dopamine release (Javitt *et al.*, 2000) and PCP-induced hyperactivity in rodents (Javitt *et al.*, 1997, 1999; Toth *et al.*, 1986). Studies have been performed with selective, high-affinity GTIs such as *N*[3-(4′-fluorophenyl)-3-(4′-phenylphenoxy)propyl]sarcosine (NFPS; Aubrey and Vandenberg, 2001), Org 24598 (Brown *et al.*, 2001), CP-802,079 (Martina *et al.*, 2004), or SSR504734 (Depoortere *et al.*, 2005).

As with GDA, high-affinity GTIs have been found to reverse PCP-induced hyperactivity (Harsing *et al.*, 2003) and dopaminergic hyperreactivity (Javitt *et al.*, 2004) in rodents, and to potentiate NMDA responses in hippocampal slices *in vitro* (Bergeron *et al.*, 1998; Depoortere *et al.*, 2005; Kinney *et al.*, 2003b; Martina *et al.*, 2004) and prefrontal cortical neurons *in vivo* (Chen *et al.*, 2003). Glycine transport inhibitors also reverse PPI abnormalities in DBA/2J mice (Depoortere *et al.*, 2005; Kinney *et al.*, 2003b) and rats with neonatal hippocampal lesions (Le Pen *et al.*, 2003), supporting a potential role of GTIs in treatment of schizophrenia. In striatal dopamine assays, GLYT1 inhibitors reduce amphetamine-induced dopamine release *in vivo* (Javitt *et al.*, 2004) and NMDA-stimulated release *in vitro* (Bennett and Gronier, 2005; Javitt *et al.*, 2005b), suggesting a likely effect on positive, as well as negative, symptoms of schizophrenia.

At saturating doses, effective GLYT1 inhibitors produce approximately two- to threefold increases in extracellular glycine concentrations (Depoortere *et al.*, 2005; Martina *et al.*, 2004). Significantly, however, positive effects on NMDA receptor-mediated neurotransmission occur at concentrations two to three orders of magnitude lower than those needed to significantly increase extracellular glycine levels. Further, an inverted U-shape curve has been observed in several studies where beneficial effects of GLYT1 antagonists on NMDA function may be diminished or lost at the highest doses used. These findings are consistent with a model in which GLYT1 inhibitors primarily affect glycine concentrations within the synaptic cleft (Fig. 2), which represents a separate brain compartment from the overall extracellular space or cerebrospinal fluid. Increases in extracellular glycine levels would occur only as a consequence of diffusion of glycine from

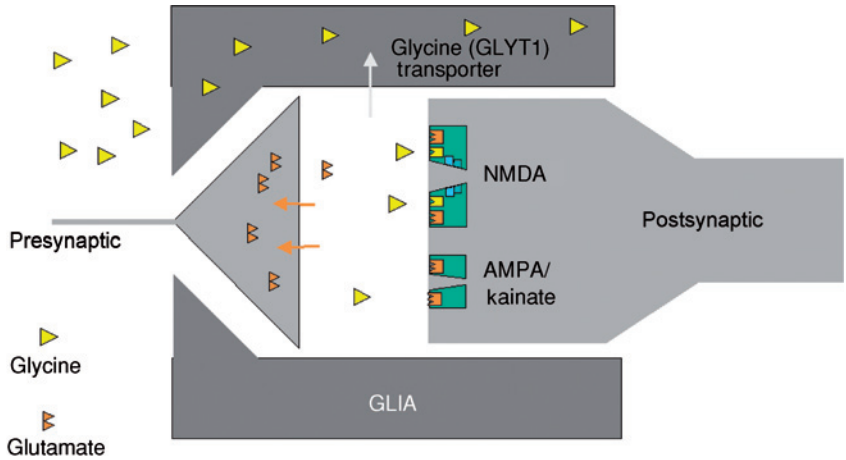

FIG. 2. Schematic model of synaptic glycine regulation by glycine transport inhibitors.

the synaptic to the general extracellular space, a process that would occur only at very high glycine concentrations. At extremely high concentrations, glycine may induce internalization of NMDA receptors, leading to loss of facilitatory glycine effects on NMDA transmission (Martina *et al.*, 2004). In rodents, effects of GLYT1 inhibitors have been found to be similar to those of clozapine (Lipina *et al.*, 2005), suggesting potential overlap of cognition enhancing mechanisms.

Further support for use of GLYT1 antagonists comes from studies of GLYT1 knockdown mice. GLYT1$^{-/-}$ mice cannot be developed due to neonatal lethality (loss of breathing) (Tsai *et al.*, 2004b). Nevertheless, alternative strategies have been employed. For example, GLYT1$^{+/-}$ heterozygote mice show a significant decrease in GLYT1 expression throughout brain and an enhancement of hippocampal NMDA activity consistent with GLYT1 inhibitor studies (Gabernet *et al.*, 2005; Martina *et al.*, 2005; Tsai *et al.*, 2004b). Similarly, selective forebrain knockouts show reductions in frontal glycine transport and potentiation of hippocampal NMDA responses as well as procognitive ability on several learning/memory paradigms (Yee *et al.*, 2006). As with animals treated with GLYT1 inhibitors, GLYT1$^{+/-}$ heterozygote mice show some evidence of NMDA internalization, particularly behavioral hypersensitivity to the NMDA antagonist MK-801 (Tsai *et al.*, 2004b). Nevertheless, on balance, the phenotype supports a net potentiation of NMDA neurotransmission.

Sarcosine (*N*-methylglycine) is a naturally occurring GLYT1 antagonist that has been used for preliminary proof-of-principle studies. To date, clinical studies with sarcosine have been conducted only in Taiwan due to its regulatory status in

the United States. In one study (Tsai *et al.*, 2004a) in which it was combined with mixed typical and atypical antipsychotics, sarcosine, at a dose of 30 mg/kg/day (2.1 g/day) produced clinical effects extremely similar to those of glycine and D-serine (Table I). In contrast, when combined with clozapine (Lane *et al.*, 2006), sarcosine had no significant effect on symptoms, also consistent with prior glycine/D-serine studies. Interestingly, in one study where sarcosine and D-serine were added to risperidone in acutely relapsing subjects.

C. Other Ionotropic Targets

Because AMPA receptors function in concert with NMDA receptors, they have been proposed as alternative therapeutic targets in schizophrenia. AMPAkines function as positive allosteric modulators of AMPA receptor-mediated neurotransmission, and facilitate learning and memory in both human (Ingvar *et al.*, 1997) and animal (Hampson *et al.*, 1998a,b) models. Further, these drugs act synergistically with antipsychotics to reverse amphetamine-induced hyperactivity (Johnson *et al.*, 1999).

In a pilot study, the AMPAkine CX-516 induced significant improvements in memory and attention when added to clozazpine, despite lack of symptomatic improvement (Goff *et al.*, 2001). However, these results were not confirmed in a larger confirmation study (Goff *et al.*, 2005), nor were beneficial effects observed in a small monotherapy trial (Marenco *et al.*, 2002). Although downregulation of AMPA receptors is less with AMPAkines than with direct agonists, there is some concern that downregulation may nonetheless occur and may limit long-term treatment strategies (Jourdi *et al.*, 2005).

Lamotrigine, an antiepileptic that reduces presynaptic glutamate release, has also been proposed as a potential adjunctive medication in schizophrenia, based on the theory that detrimental effects of NMDA antagonists on cognitive functioning may be due to glutamatergic rebound (Anand *et al.*, 2000; Dursun *et al.*, 1999). In a clinical challenge study, lamotrigine prevented acute psychotomimetic effects of ketamine, with greater effects on positive than negative symptoms (Anand *et al.*, 2000) supporting potential therapeutic efficacy. Improvements in positive and general symptoms were reported as well in small-scale studies of lamotrigine in clozapine-treated patients with persistent clinical symptoms (Dursun and Deakin, 2001; Tiihonen *et al.*, 2003). However, effects failed to reach statistical significance in a subsequent double blind study (Kremer *et al.*, 2004). Further, an industry-sponsored multicenter controlled study also did not show significant benefit (http://ctr.gsk.co.uk/Summary/lamotrigine/studylist.asp). Thus, as of yet limited clinical evidence is available to support the efficacy of either AMPA agonists or general glutamate antagonists.

D. Metabotropic Receptors

Metabotropic modulators are currently in an early stage of development for treatment of schizophrenia. Studies attempting to validate metabotropic receptors as therapeutic targets in schizophrenia have been based on two alternative conceptualizations of the disorder. Group I receptors potentiate presynaptic glutamate release and NMDA receptor-mediated neurotransmission. Therapeutic effectiveness of Group I agonists is therefore predicted based on models which postulate low NMDA receptor activity and/or glutamate levels as being pathophysiological in schizophrenia. In contrast, Group II/III agonists inhibit glutamate release. Use of these agents follows models, which postulate that glutamatergic hyperactivity may be pathophysiological.

E. Group I Receptors

Group I includes both mGLUR1 and mGLUR5 receptors, both of which stimulate NMDA receptors via differential second messenger cascades (Benquet *et al.*, 2002; Heidinger *et al.*, 2002). Preclinical studies have evaluated the ability of Group I antagonists to induce schizophrenia-like behavioral effects, and Group I agonists to reverse effects of amphetamine, PCP and other psychotomimetics. The most widely used mGluR5 antagonist, 2-methyl-6-(phenylethynyl)pyridine (MPEP), does not affect locomotor activity or PPI by itself but potentiates PCP-induced increases in locomotor activity and disruption of PPI (Henry *et al.*, 2002; Kinney *et al.*, 2003a). Similar effects have been observed with the more recently develop compound 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]-pyridine (MTEP) (Cosford *et al.*, 2003; Pilc *et al.*, 2002). Finally, mGluR1 (Brody *et al.*, 2003a) and mGluR5 (Kinney *et al.*, 2003a) knockout mice show disruptions of PPI, that respond poorly to known treatments for schizophrenia (Brody *et al.*, 2003b), supporting a potential role of Group I receptors as therapeutic targets in schizophrenia. Group I antagonists also produce anxiolytic-like effects in several animal models of anxiety, suggesting that they may be independent targets for the treatment of anxiety disorders (Chojnacka-Wojcik *et al.*, 2001).

Studies with Group I agonists have also been supportive of potential therapeutic effectiveness, but are more limited. For example, the mGluR5 agonist 2-chloro-5-hydroxyphenylglycine (CHPG) has been found to reverse PPI-disruptive effects of amphetamine in rodents (Kinney *et al.*, 2003a). Similarly, both nonselective and Group I selective agonists inhibit PCP-induced dopamine release in rodent prefrontal cortex (Maeda *et al.*, 2003). An issue in the use of direct agonists is rapid receptor desensitization, preventing chronic use. An alternative approach is the use of positive allosteric modulators, which, do not bind directly to

the agonist-binding site. Positive modulators, in general, have proven to be lipophilic and centrally acting, making them attractive as potential pharmacological agents (Pin and Acher, 2002).

Despite some encouraging results with Group I agonists in animal models, clinical data remain lacking. Further, Group I receptors have a markedly different cellular distribution in primates than rodents (Muly *et al.*, 2003; Paquet and Smith, 2003). Thus, primate studies and eventual clinical trials will be needed to validate this target for treatment of neuropsychiatric disorders.

F. Group II Metabotropic Agonists

Groups II and III metabotropic receptors are negatively linked to glutamate release, and may limit endogenous release under conditions of glutamate excess. Use of Group II/III agonists in schizophrenia is therefore based on the hypothesis that increased glutamate levels may be pathophysiological. Several high-affinity agonists have been developed over recent years, including (−)-2-oxa-4-amino-bicyclo[3.1.0.]hexane-4,6-dicarboxylate (LY379268) and the related compound LY354740, permitting characterization of effects of Group II agonists in both preclinical and clinical studies (Schoepp and Marek, 2002).

An initial study with LY379268 demonstrated its ability to block PCP-induced increases in prefrontal glutamate, along with PCP-induced impairments in working memory, suggesting a role of glutamatergic hyperactivity in at least some forms of prefrontal dysfunction (Lorrain *et al.*, 2003; Moghaddam and Adams, 1998). Similarly, LY3279268 has been shown by a variety of groups to inhibit PCP-induced hyperactivity during both acute (Clark *et al.*, 2002; Makino *et al.*, 2003) and repeated (Cartmell *et al.*, 2000) administration, and reverse PCP-induced behaviors in monoamine depleted mice (Swanson and Schoepp, 2002). Finally, LY354740 has been found to reverse effects of NMDA antagonists in both rodents and humans (Krystal *et al.*, 2004; Moghaddam and Adams, 1998). Despite these intriguing results, however, clinical data with metabotropic agonists or antagonists are yet to be reported.

V. Potential Causes of Glutamatergic Dysfunction in Schizophrenia

The observation that NMDA antagonists induce both symptoms and neurocognitive deficits closely resembling those of schizophrenia (at least the early stages), suggests strongly that dysfunction or dysregulation of NMDA receptor-mediated neurotransmission may contribute heavily to the pathophysiology of schizophrenia. As of yet, however, the basis for NMDA dysfunction has yet to be

determined. Given dopaminergic theories of the disorder, one issue concerns whether glutamatergic deficits in schizophrenia may be secondary to primary disturbances in dopaminergic mechanisms. Additionally, schizophrenia is generally conceived as having both genetic and environmental components, with each set of factors contributing approximately half to overall risk. Over recent years, candidate genetic and environment mechanisms have been proposed. Several of these mechanisms significantly impact glutamatergic neurotransmission, and may account for the apparent NMDA dysregulation observed in schizophrenia.

A. Dopamine–Glutamate Interactions

At present, dopamine D2 antagonists are the mainstay treatments for schizophrenia. Their efficacy depends on the presumed dysregulation of dopaminergic systems in schizophrenia, although objective evidence of such dysregulation is observed primarily during acute phases of the illness (Laruelle *et al.*, 1999). As such, the issue arises as to how dopaminergic treatments affect NMDA receptor-mediated phenomena, and whether primary disturbances in dopaminergic neurotransmission may contribute to secondary dysregulation of NMDA activation.

The strongest evidence for primary dopaminergic dysfunction in schizophrenia, at present, comes from studies of polymorphisms of the catechol-*O*-methyltransferase (COMT) gene. A common SNP in the COMT gene causes a Val to Met transition at AA158/AA108 (Val158Met), resulting in reduced COMT activity in Met allele carriers, and thus increased dopamine levels in prefrontal cortex. In initial studies, increased presence of the Val (high activity) allele was associated with increased risk for schizophrenia as well as impaired prefrontal function (Weinberger *et al.*, 2001), suggesting that low prefrontal dopamine levels may mediate both sets of effect.

However, considerable controversy has arisen concerning the linkage to schizophrenia (Meyer-Lindenberg *et al.*, 2006), with several studies failing to find an association with schizophrenia independent of effects on cognition (Ehlis *et al.*, 2006). It has also been suggested that COMT polymorphisms are not associated with schizophrenia itself, but with manic (Derosse *et al.*, 2006), as well as aggression (Lachman *et al.*, 1998) symptoms within schizophrenia, which may lead to overrepresentation in specific clinical populations. To the extent that COMT polymorphisms are associated with schizophrenia, they suggest that low, rather than high, dopamine levels in PFC may be pathogenic. Whether the association with schizophrenia is ultimately supported, however, remains to be determined.

Other evidence for potential etiological involvement of dopamine in schizophrenia derives from convergences between brain glutamatergic and dopaminergic systems. One primary site of convergence of glutamatergic and dopaminergic systems is on dendritic shafts and spines of striatal GABAergic medium spiny

interneurons (for review see Kotter, 1994; Smith and Bolam, 1990; Starr, 1995). In striatum, NMDA and D2 receptors produce opposite effects, with NMDA receptors producing net stimulation of striatal interneurons, and D2 receptors producing net inhibition (Cepeda and Levine, 1998; Cepeda *et al.*, 2001; Leveque *et al.*, 2000; Nicola *et al.*, 2000; Onn *et al.*, 2000; Peris *et al.*, 1988; West and Grace, 2002). Thus, in striatum, NMDA antagonists and dopamine agonists produce similar inhibition of GABAergic outflow, the first by decreasing excitation and the second by increasing inhibition.

Dopamine is not needed to mediate the behavioral effects of NMDA antagonists in rodents (Chartoff *et al.*, 2005). Nevertheless, dopamine may enhance these effects, and D2 antagonists may normalize GABAergic tone in striatum regardless of whether the primary deficit consists of dopaminergic hyperactivity or glutamatergic hypoactivity. Both glycine and GTIs have been shown to increase NMDA-stimulated GABA release in isolated rodent striatum, while decreasing NMDA-stimulated dopamine release (Javitt *et al.*, 2005b), consistent with a facilitatory effect on NMDA activation of GABAergic striatal interneurons. GABAergic effects, in turn, are mediated most likely through $GABA_B$ receptors on presynaptic dopaminergic terminals (Javitt *et al.*, 2005b).

In contrast to D2 receptors, which mediate opposite effects to those of NMDA, D1 receptors show complex but primarily facilitatory interactions (Cepeda and Levine, 2006; Missale *et al.*, 2006). First, D1 receptors potentiate NMDA receptor-mediated responses via stimulation of cyclic AMP (cAMP)-protein kinase A pathway leading to DARPP-32 phosphorylation and subsequent phosphorylation of the NMDA receptor. Second, D1 reception stimulation induces translocation of NMDA supporting a potential therapeutic role of D1 agonists. Second, D1 receptor activation results in rapid translocation of NMDA receptors to postsynaptic membranes, which, in turn, recruits D1 receptors to the membrane and enhances D1 receptor-mediated cAMP, leading to a positive feedback loop. Finally, direct protein–protein coupling between D1 and NMDA receptors may occupy, primarily involving NR1 and NR2A, but not NR2B, subunits. The interaction between D1 and NR2A receptors may attenuate NMDA response. Thus, depending on which effects predominate, either D1 agonists or antagonists might produce psychotherapeutic effects in schizophrenia.

Convergence between D1 and NMDA receptors occurs as well in cortex, where D1 receptors predominate over D2. In rodents and primates, chronic exposure to the NMDA receptors antagonists PCP and MK-801 results in decreased DA levels in the PFC (Jentsch and Roth, 1999; Jentsch *et al.*, 1997, 1998; Tsukada *et al.*, 2005). Further, in monkeys (Jentsch and Roth, 1999; Jentsch *et al.*, 1997, 1998; Tsukada *et al.*, 2005), chronic exposure to the NMDA antagonist MK-801 has been found to gradually lower DA levels in the PFC, and gradually upregulate binding of the D1 ligand [^{11}C]NNC 112. This finding was highly reminiscent of the increased

[^{11}C]NNC 112 binding observed in patients with schizophrenia in the DLPFC (Abi-Dargham *et al.*, 2002).

Furthermore, in the Tsukada *et al.* (2005) monkey study, upregulated prefrontal [^{11}C]NNC 112 BP was associated with impaired WM performance, a relationship observed in patients with schizophrenia as well (Abi-Dargham *et al.*, 2002). These data supported the hypothesis that, in schizophrenia, increased [^{11}C]NNC 112 BP is a compensatory response to a sustained deficit in prefrontal DA function stemming from a sustained deficit in NMDA transmission. Of note, however, alterations in D1 binding in both monkeys (Jentsch and Roth, 1999; Jentsch *et al.*, 1997, 1998; Tsukada *et al.*, 2005) and humans (Abi-Dargham *et al.*, 2002) are restricted to prefrontal cortex, suggesting that such interactions cannot easily account for cognitive deficits in schizophrenia that localize elsewhere in brain. In cortex, D2 receptor stimulation inhibits NMDA responses similarly to that observed in striatum (Tseng and O'Donnell, 2004). Although D2 receptor density in cortex is generally low, this interaction would be consistent with procognitive effects of D2 antagonists under hypofunctional NMDA conditions.

A final site of convergence is through glutamatergic projections from prefrontal cortex to midbrain dopaminergic nuclei. These projections modulate activity of midbrain DA neurons via an activating pathway, which has been termed the "accelerator" and an inhibitory pathway that has been called the "brake." This dual excitatory–inhibitory interaction potentially permits the prefrontal cortex to fine-tune dopaminergic activity and produce regionally dichotomatous effects. AMPA and NMDA receptors also serve different roles in this system, with AMPA receptors subserving tonic inhibitory regulation of mesoaccumbens neurons and a tonic excitatory regulation of mesoprefrontal DA neurons, and NMDA receptors mediated primarily phasic responses to behaviorally relevant stimuli (Takahata and Moghaddam, 2000). Loss of this descending glutamatergic input thus could produce subcortical dopaminergic hyperactivity and prefrontal hypoactivity, similar to what has been postulated to occur in schizophrenia.

Overall, therefore, dopamine–glutamate interactions remain an area of active research. To date, other than COMT linkages, little evidence implicates intrinsic dopaminergic deficits in the pathophysiology of schizophrenia, although both D1 and D2 receptors are part of interactive cascades that may potently modulate glutamatergic function. D1 agonists, in particular, have been suggested as potential psychotherapeutic agents based on their ability to potentiate NMDA responses (Goldman-Rakic *et al.*, 2004). However, clinical data supporting this hypothesis to date remain lacking. Further, based on patterns of cognitive change in COMT genotypes and patterns of D1 alteration in schizophrenia, D1 agonist effects may be limited to prefrontal-type deficits, and may fail to affect more distributed aspects of neurocognitive dysfunction in schizophrenia.

B. Linkage–Association Studies in Schizophrenia

Adoption studies of schizophrenia suggest that ~50% of the risk for schizophrenia is genetic, with the other 50% being attributable to environmental factors. Candidate genes for schizophrenia have only recently been identified, however, and considerable controversy continues to surround many of the targets. Nevertheless, a consistent and somewhat surprising finding (to the uninitiated) from genetic studies in schizophrenia is that several of the identified genes interact closely with glutamatergic mechanisms in general, and NMDA receptors in particular. As such, these studies provide additional support for glutamatergic theories of the disorder.

One of the best established candidate genes for schizophrenia is neuregulin, a brain transmitter that mediates its effects primarily through ErbB3 and ErbB4 receptors. A positive linkage to the 8p locus encoding the neuregulin 1 (NRG1) gene was first reported in 2002 (Stefansson *et al.*, 2002). A meta-analysis of 13 studies published through November, 2005 confirmed the association, suggesting an odd ratio of 1.22 and a p value of $<10-9$. Linkages to polymorphisms in the gene encoding the ErbB4 receptor, particularly in Ashkenazi Jews, have also been reported (Silberberg *et al.*, 2006). In initial studies, it was suggested that NRG1 might mediate its risk-enhancing effects based on interaction with NMDA receptors, based on the observation that NRG1 hypomorphs had fewer functional NMDA receptors than wild-type mice (Stefansson *et al.*, 2002). In a recent functional postmortem study, NRG1 stimulation was found to suppress NMDA receptor activation in prefrontal cortex tissue from schizophrenia patients to a greater extent than it did in tissue from matched comparison subjects (Hahn *et al.*, 2006). Thus, increased expression of NRG1 may increase risk for schizophrenia primarily by downregulating cortical NMDA receptor-mediated neurotransmission.

Two other genes with strong relationship to schizophrenia, D-amino acid oxidase (DAAO) and G72 (aka DAOA), have also been linked to schizophrenia in at least some studies. DAAO is the primary enzyme responsible for degradation of D-serine in brain. G72 is a modulatory subunit for DAAO that appears to have arisen during late primate evolution. An initial linkage of both genes to schizophrenia was first reported in 2002 in a Russian cohort (Chumakov *et al.*, 2002), with the most active combination of DAAO and G72 (i.e., the forms that would produce lowest D-serine levels) producing the greatest risk for developing schizophrenia. These genetic findings resonate with independent neurochemical findings of decreased CSF D-serine levels in schizophrenia (Hashimoto *et al.*, 2005). The DAAO and G72 findings were subsequently confirmed in an independent German sample (Schumacher *et al.*, 2004), and the G72 finding in an Ashkenazi cohort (Korostishevsky *et al.*, 2004). In contrast, no association with either gene was found in a recently studied Taiwanese cohort (Liu *et al.*, 2006). A study by Goldberg *et al.* (2006) also did not find significant associations of

DAAO or G72 and schizophrenia, although G72 was strongly associated with cognitive dysfunction and reduced hippocampal activation during an episodic working memory task. Thus, whether polymorphisms of DAAO and G72 explain increased risk for schizophrenia, they may be associated with accompanying neurocognitive dysfunction.

To date, there is also limited evidence implicating NMDA receptors directly in the genetics of schizophrenia. One positive linkage study was reported in an African Bantu population but has not been replicated (Riley *et al.*, 1997). Studies have also reported some linkages between NR2 subunits and either schizophrenia itself (Di Maria *et al.*, 2004) or with specific clinical features of the disorder (Chiu *et al.*, 2003; Itokawa *et al.*, 2003). Other potential risk genes for schizophrenia such as dysbindin (DTNBP1), disrupted in schizophrenia-1 (DISC-1), RGS4, and metabotropic glutamate receptor 3 (GRM-3) may also converge on glutamatergic systems (Harrison and Weinberger, 2005; Moghaddam, 2003; Weinberger, 2005), although further clarification is needed concerning functional consequences of risk haplotypes for brain function.

C. Environmental and Neurochemical Factors

Finally, environmental factors that contribute to development of schizophrenia may also converge on NMDA receptors. For example, it has been hypothesized that perinatal hypoxia, an important risk factor for schizophrenia, leads to neurotoxic degeneration of NMDA-bearing cells, an effect that may only produce behavioral symptoms later in development (Olney *et al.*, 1999). Similarly, schizophrenia has been associated with decreased plasma levels of the NMDA agonists glycine (Sumiyoshi *et al.*, 2004) and D-serine (Hashimoto *et al.*, 2003), and increased levels of homocysteine (Levine *et al.*, 2002; Susser *et al.*, 1998), an agent that may act as a functional NMDA antagonist. Levels of kynurenic acid, an endogenous NMDA and nicotine receptor antagonist, may also be high in schizophrenia (Erhardt *et al.*, 2001; Schwarcz *et al.*, 2001) and lead to inhibition of glutamatergic/NMDA function.

A final compound of potential etiological interest in schizophrenia is glutathione. Glutathione regulates NMDA receptors at the redox site. Low glutathione levels have been reported in CSF and prefrontal cortex in schizophrenia *in vivo* (Do *et al.*, 2000). In hippocampal slices, reduced glutathione levels are associated with reduced presynaptic glutamate release along with postsynaptic NMDA activity, consistent with the phenotype observed in schizophrenia (Steullet *et al.*, 2006). Although determinants of various neurochemical levels in brain are unknown at present, present findings suggest that alterations in metabolism or environmental exposure may explain significant variance in risk for developing schizophrenia, along with genetic factors.

VI. Future Research and Treatment Implications

Over the last 40 years, the dopamine model has been the leading neurochemical hypothesis of schizophrenia. This model has proven heuristically valuable, with all current medications for schizophrenia functioning primarily to block dopamine D2 receptors. Yet it remains unlikely that dopaminergic dysfunction, on its own, can fully account for the wide range of symptoms and neurocognitive deficits seen in schizophrenia. Glutamatergic models provide an alternate approach for conceptualizing the brain abnormalities associated with schizophrenia. As opposed to dopaminergic agonists, NMDA antagonists produce negative and cognitive symptoms of schizophrenia, along with positive symptoms, and induce neuropsychological deficits that are extremely similar to those observed in schizophrenia. At present, there are no approved medications for treatment of either negative symptoms or neurocognitive dysfunction. New treatment approaches aimed at potentiating glutamatergic neurotransmission particularly at NMDA receptors, however, offer some new hope for future clinical development.

Acknowledgments

Preparation of this chapter was supported in part by USPHS grants K02 MH01439, R01 DA03383, and R37 MH49334, and by a Clinical Scientist Award in Translational Research from the Burroughs Wellcome Fund.

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DECIPHERING THE DISEASE PROCESS OF SCHIZOPHRENIA: THE CONTRIBUTION OF CORTICAL GABA NEURONS

David A. Lewis*,† and Takanori Hashimoto*

*Department of Psychiatry, University of Pittsburgh, Pennsylvania 15213, USA
†Department of Neuroscience, University of Pittsburgh, Pennsylvania 15213, USA

Schizophrenia is a devastating illness that is manifest through a variety of clinical signs and symptoms. Among these, impairments in certain critical cognitive functions, such as working memory, appear to represent the core features of the disorder. In this chapter, we review the evidence indicating that disturbances in neurotransmission by a subset of GABA neurons in the dorsolateral prefrontal cortex are commonly present in schizophrenia. Despite both pre- and postsynaptic compensatory responses, the resulting pathophysiological process, alterations in the perisomatic inhibitory regulation of pyramidal neurons, underlies a reduced capacity for the synchronization of neuronal activity at gamma frequencies that is required for working memory function. We also discuss several pathogenetic mechanisms that could rise to the alterations in GABA neurotransmission and consider the implication of these findings for therapeutic interventions to improve cognitive function in individuals with schizophrenia.

I. Working Memory Impairments: A Core Feature of Schizophrenia

Of the multiple clinical features of schizophrenia, disturbances in certain cognitive processes, such as impairments in attention, some types of memory, and executive function, appear to represent core features of the illness (Elvevåg

INTERNATIONAL REVIEW OF NEUROBIOLOGY, VOL. 78
DOI: 10.1016/S0074-7742(06)78004-7

0074-7742/07 $35.00

and Goldberg, 2000). Cognitive abnormalities have been observed during the premorbid and prodromal phases of the illness (Davidson *et al.*, 1999), at the initial onset of psychosis (Saykin *et al.*, 1994), and throughout the later stages of the illness (Heaton *et al.*, 1994). Perhaps most important, the degree of cognitive impairment is the best predictor of long-term outcome in individuals with schizophrenia (Green, 1996).

At least some of the critical cognitive deficits in schizophrenia reflect alterations in working memory (the ability to transiently maintain and manipulate a limited amount of information in order to guide thought or behavior) that is mediated by the circuitry of the dorsolateral prefrontal cortex (DLPFC) (Miller and Cohen, 2001). Many individuals with schizophrenia perform poorly on working memory tasks and exhibit altered activation of the DLPFC when attempting to perform such tasks (Callicott *et al.*, 2003; Perlstein *et al.*, 2001; Weinberger *et al.*, 1986). In contrast, these abnormalities have not been found in individuals with other psychotic disorders (MacDonald *et al.*, 2005) or major depression (Barch *et al.*, 2003). The altered activation of the DLPFC during working memory tasks predicts the severity of cognitive disorganization symptoms in subjects with schizophrenia (Perlstein *et al.*, 2001), and reduced working memory capacity has been suggested to be rate limiting in the performance of other cognitive tasks in schizophrenia (Silver *et al.*, 2003).

II. Working Memory Impairments and Altered GABA Neurotransmission in the DLPFC

Working memory depends on the coordinated and sustained firing of subsets of DLPFC pyramidal neurons between the temporary presentation of a stimulus cue and the later initiation of a behavioral response (Goldman-Rakic, 1995). Although other neurotransmitter systems are also involved, inhibitory signaling via γ-aminobutyric acid (GABA) appears to be critical for this pattern of activity in DLPFC pyramidal neurons during working memory. Fast-spiking GABA neurons in monkey DLPFC are active during the delay period of working memory tasks (Wilson *et al.*, 1994) and are necessary for task-related firing and the spatial tuning of pyramidal neurons during working memory (Rao *et al.*, 2000). In addition, the injection of GABA antagonists in the DLPFC disrupts working memory performance (Sawaguchi *et al.*, 1989). Thus, these findings suggest that disturbances in GABA neurotransmission in the DLPFC could contribute to the working memory impairments in schizophrenia.

Consistent with this hypothesis, markers of GABA neurotransmission are altered in the DLPFC of subjects with schizophrenia. For example, reduced expression of the mRNA for the 67-kDa isoform of glutamic acid decarboxylase (GAD_{67}), an enzyme that synthesizes GABA, (Akbarian *et al.*, 1995; Guidotti

et al., 2000; Hashimoto *et al.*, 2005b; Mirnics *et al.*, 2000; Vawter *et al.*, 2002; Volk *et al.*, 2000) is one of the most consistent findings in postmortem studies of individuals with schizophrenia (Torrey *et al.*, 2005). The only exception to this finding was reported in one cohort of elderly, chronically hospitalized individuals with schizophrenia (Dracheva *et al.*, 2004). Although less extensively studied, the deficit in GAD_{67} mRNA appears to be accompanied by a corresponding decrease in the cognate protein (Guidotti *et al.*, 2000). In contrast, both the overall protein and mRNA expression levels (Guidotti *et al.*, 2000) of another synthesizing enzyme for GABA, GAD_{65}, and the density of GAD_{65}-immunoreactive axon terminals (Benes *et al.*, 2000) were reported to be unchanged in the DLPFC of subjects with schizophrenia. Interestingly, elimination of the GAD_{65} gene in mice has a limited effect on cortical levels of GABA, whereas genetically engineered reductions in GAD_{67} mRNA expression are associated with profound decreases in cortical GAD activity and GABA content (Asada *et al.*, 1997).

In the DLPFC of subjects with schizophrenia, GAD_{67} mRNA expression is undetectable in a subpopulation (about 25–30%) of GABA neurons (Akbarian *et al.*, 1995; Volk *et al.*, 2000), whereas the majority of GABA neurons have expression levels of GAD_{67} mRNA that do not differ from normal comparison subjects (Volk *et al.*, 2000). Furthermore, in the same individuals, the mRNA expression for the GABA membrane transporter (GAT1), a protein responsible for reuptake of released GABA into nerve terminals, is similarly decreased in a subpopulation of GABA neurons (Volk *et al.*, 2001). The affected GABA neurons appear to be principally located in cortical layers 2–5; neither GAD_{67} nor GAT1 mRNA expression is altered in layer 6. Together, these findings suggest that both the synthesis and reuptake of GABA are greatly reduced in a subset of DLPFC inhibitory neurons in schizophrenia.

The affected GABA neurons include those that contain the calcium-binding protein parvalbumin (PV), which is present in ~25% of GABA neurons in the primate DLPFC (Condé *et al.*, 1994), as demonstrated by the decreased expression of PV mRNA in layers 3 and 4, but not in layers 2, 5, or 6, of the DLPFC in subjects with schizophrenia (Hashimoto *et al.*, 2003). However, in contrast to the findings for GAD_{67} and GAT1 mRNAs, the density of neurons with detectable levels of PV mRNA was not changed in subjects with schizophrenia, but the expression level of PV mRNA per neuron was significantly decreased. In addition, within the same subjects, the expression level of PV mRNA per neuron was strongly correlated with the change in density of GAD_{67} mRNA-positive neurons. Furthermore, dual label *in situ* hybridization studies demonstrated that approximately half of PV mRNA-positive neurons in subjects with schizophrenia lacked detectable levels of GAD_{67} mRNA (Hashimoto *et al.*, 2003). Finally, these findings were consistent with the results of immunocytochemical studies that reported similar densities of PV-immunoreactive neurons in the DLPFC of normal comparison and schizophrenia subjects (Beasley *et al.*, 2002;

Woo *et al.*, 1997). Thus, PV-containing GABA neurons are not reduced in number in the DLPFC of subjects with schizophrenia, but they do exhibit reduced expression of several critical genes, indicating that they are present but functionally impaired. In contrast, the expression of the mRNA for calretinin, a calcium-binding protein present in ~50% of GABA neurons in the primate DLPFC (Condé *et al.*, 1994), is not altered in schizophrenia (Hashimoto *et al.*, 2003), nor is the density of calretinin-immunoreactive neurons (Daviss and Lewis, 1995) or axon terminals (Woo *et al.*, 1997).

In addition to their calcium-binding protein content, PV-containing neurons are distinguishable from other cortical GABA neurons (Fig. 1) by their firing patterns, preferred synaptic targets, and morphological features (Kawaguchi, 1995). For example, in macaque monkey DLPFC, cluster analysis of multiple physiological features revealed that PV-containing neurons are distinctly different from all other types of GABA neurons (Krimer *et al.*, 2005; Zaitsev *et al.*, 2005). Furthermore, PV-positive neurons in the primate DLPFC are composed of two morphologically distinct subtypes (Condé *et al.*, 1994). Chandelier (or axoaxonic) neurons furnish a linear array of axon terminals (termed cartridges) that synapse exclusively on the axon initial segment of pyramidal neurons (Somogyi, 1977), whereas the axons of wide-arbor (basket) neurons have a much larger spread than those of chandelier cells and their axon terminals principally target the cell body and proximal dendrites of pyramidal neurons (Lewis and Lund, 1990). Both of these types of PV-containing neurons have indistinguishable fast-spiking, nonadapting patterns of firing (González-Burgos *et al.*, 2005). The proximity of the perisomatic inhibitory synapses formed by PV-containing chandelier and wide-arbor neurons to the site of action potential generation in pyramidal neurons suggests that these GABA neurons are specialized to powerfully regulate the output of pyramidal neurons. For example, during hippocampal oscillations *in vivo* chandelier cells exhibit maximal firing probability 180° out of phase with pyramidal neurons, indicating the ability of inhibitory inputs from chandelier neurons to facilitate the rhythmic entrainment of pyramidal cell discharge, time locking the activity of local populations of pyramidal cells to fire together (Klausberger *et al.*, 2003). In contrast, an *in vitro* study indicated that GABA inputs to the axon initial segment of pyramidal neurons could actually sufficiently depolarize pyramidal cells to fire action potentials under certain conditions (Szabadics *et al.*, 2006); however, whether this observation holds under *in vivo* conditions remains to be determined.

PV-containing GABA neurons also undergo marked and distinctive refinements in the monkey DLFPC during adolescence (Fig. 2) (Cruz *et al.*, 2003; Erickson and Lewis, 2002). These developmental trajectories suggest that PV-containing neurons contribute to the increased engagement of DLPFC circuitry in (Lewis, 1997), and improved performance of (Diamond, 2002; Luna *et al.*, 2004), working memory during adolescence, providing rationale for the hypothesis that alterations in PV-positive neurons contribute to working memory dysfunction in schizophrenia.

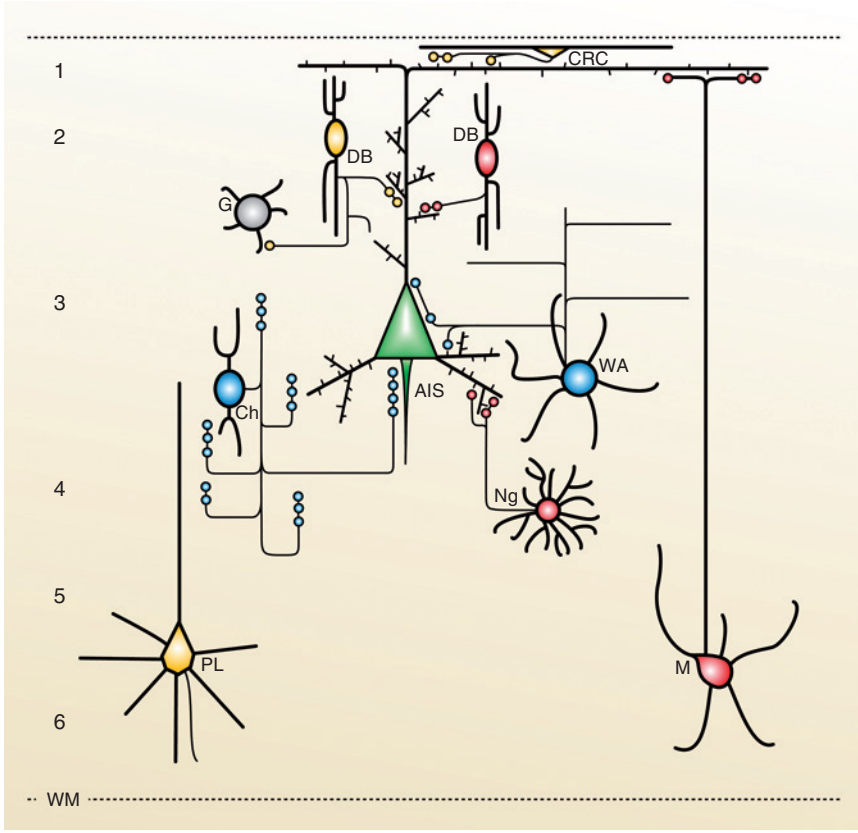

FIG. 1. Morphological and biochemical features of subclasses of cortical GABA neurons. This schematic diagram illustrates the calcium-binding protein content (blue, parvalbumin; red, calbindin; yellow, calretinin) and location of inhibitory synaptic inputs to a pyramidal neuron (green) of different morphological classes of cortical GABA neurons. The chandelier (Ch) and wide-arbor (WA) or basket neurons provide inhibitory input to the axon initial segment (AIS) or cell body and proximal dendrites, respectively, of pyramidal neurons. In contrast, the calbindin-containing double bouquet (DB), neurogliaform (Ng), and Martinotti (M) neurons tend to provide inhibitory inputs to the distal dendrites of pyramidal neurons. Finally, calretinin-containing neurons appear to target both pyramidal cell distal dendrites and other GABA (G) neurons. CRC, Cajal-Retzius cell; PL, pyramidal-like neuron. Reprinted from Lewis *et al.* (2005).

Furthermore, these developmental changes during adolescence could contribute to unmasking the consequences of inherited abnormalities in the regulation of GABA neurotransmission in schizophrenia and may help explain why certain life experiences during adolescence (e.g., stress or cannabis exposure) appear to increase the risk of the illness (Lewis and Levitt, 2002). Consistent with this

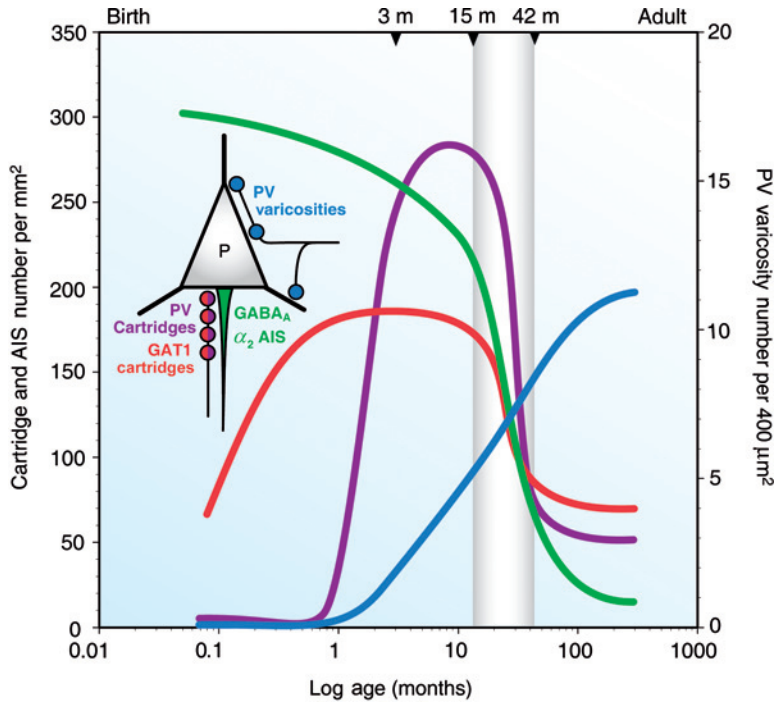

FIG. 2. Postnatal development of inputs from PV-containing GABA neurons to pyramidal neurons in monkey DLPFC. The axon terminals of chandelier neurons form vertical arrays of boutons (cartridges) that are immunoreactive for PV or GAT1 and that outline the axon initial segment of pyramidal neurons. Although the developmental time course differs somewhat for these two markers, the density of labeled cartridges is low in the DLPFC of newborn monkeys, increases to reach a peak prior to the onset of puberty, and then declines markedly during adolescence (shaded area between 15 and 42 months of age) to adult levels. These density changes in PV- and GAT-IR cartridges appear to reflect developmental shifts in the concentration of these proteins. Interestingly, the peak and subsequent decline in the density of labeled cartridges occur prior to the age when the peak density of PV-immunoreactive varicosities, putative axon terminals from the wide-arbor (basket) class of PV-containing GABA neurons is achieved. Postsynaptically, the detectability of the α_2 subunit of the $GABA_A$ receptor in pyramidal neuron axon initial segment (AIS) is high at birth, and then markedly declines during adolescence before stable adult levels are achieved. Reprinted from Lewis *et al.* (2005).

hypothesis, the density of chandelier neuron axon cartridges immunoreactive for GAT1 was significantly reduced in the DLPFC of subjects with schizophrenia (Woo *et al.*, 1998), with the effect most significant in the middle cortical layers (Pierri *et al.*, 1999). In contrast, measures of GAT1 immunoreactivity in other populations of axon terminals were unchanged (Woo *et al.*, 1998). Thus, in concert

with the observations of cell type-selective alterations in gene expression, these findings suggest that chandelier neurons in the DLPFC of subjects with schizophrenia express decreased levels of PV mRNA and undetectable levels of GAD_{67} and GAT1 mRNAs, with the latter resulting in reduced GAT1 protein in chandelier neuron axon cartridges. The potential relevance of these findings for working memory dysfunction in schizophrenia is strengthened by the failure to find such disturbances in subjects with other psychiatric disorders or in monkeys exposed chronically to antipsychotic medications in a fashion that mimics the clinical treatment of schizophrenia (Hashimoto *et al.*, 2003; Pierri *et al.*, 1999; Volk *et al.*, 2000, 2001, 2002). Furthermore, GABA levels are not altered in the prefrontal cortex of unmedicated subjects with remitted major depressive disorder, indicating that if such changes are present in symptomatic depression (as suggested from studies of the visual cortex (Sanacora *et al.*, 1999)), they are not a persistent characteristic of this illness (Hasler *et al.*, 2005).

However, the pathophysiological significance of these changes depends on how they affect GABA neurotransmission at the synapse between the chandelier neuron and the pyramidal cell axon initial segment. Specifically, do these findings reflect deficient inhibition, resulting from a primary reduction in GABA synthesis, or excessive inhibition, secondary to a reduction in GABA reuptake? Interestingly, receptors containing $GABA_A$ α_2 subunits are found principally at inhibitory synapses onto pyramidal neuron axon initial segments (Nusser *et al.*, 1996). In the DLPFC of subjects with schizophrenia, the density of pyramidal neuron axon initial segments immunoreactive for the $GABA_A$ α_2 subunit is more than double that of control subjects (Volk *et al.*, 2002), apparently reflecting higher levels of α_2 subunits in the axon initial segment, since neither the density of pyramidal neurons (Pierri *et al.*, 2003) nor of their axon initial segments (Cruz *et al.*, 2004) is increased in these same subjects. Thus, in the DLPFC of subjects with schizophrenia, $GABA_A$ receptors seem to be upregulated at pyramidal neuron axon initial segments in response to deficient GABA release from chandelier neuron axon terminals (Fig. 3).

Consistent with this interpretation, the reduced presynaptic levels of PV and GAT1 in chandelier cells appear to represent compensatory responses to a deficit in GABA release. For example, a reduction in PV would be expected to be associated with increased GABA release since, by buffering presynaptic Ca^{2+} transients, PV reduces the Ca^{2+}-dependent facilitation of GABA release during periods of repetitive firing (Vreugdenhil *et al.*, 2003). Similarly, reduced levels of GAT1 would be expected to prolong the duration of inhibitory postsynaptic currents (IPSCs) when neighboring synapses are activated synchronously (Overstreet and Westbrook, 2003). Thus, the combination of reduced presynaptic levels of PV and GAT1 proteins in chandelier axon cartridges and the postsynaptic upregulation of $GABA_A$ receptors at the axon initial segment of pyramidal neurons in the DLPFC of subjects with schizophrenia could act synergistically to increase

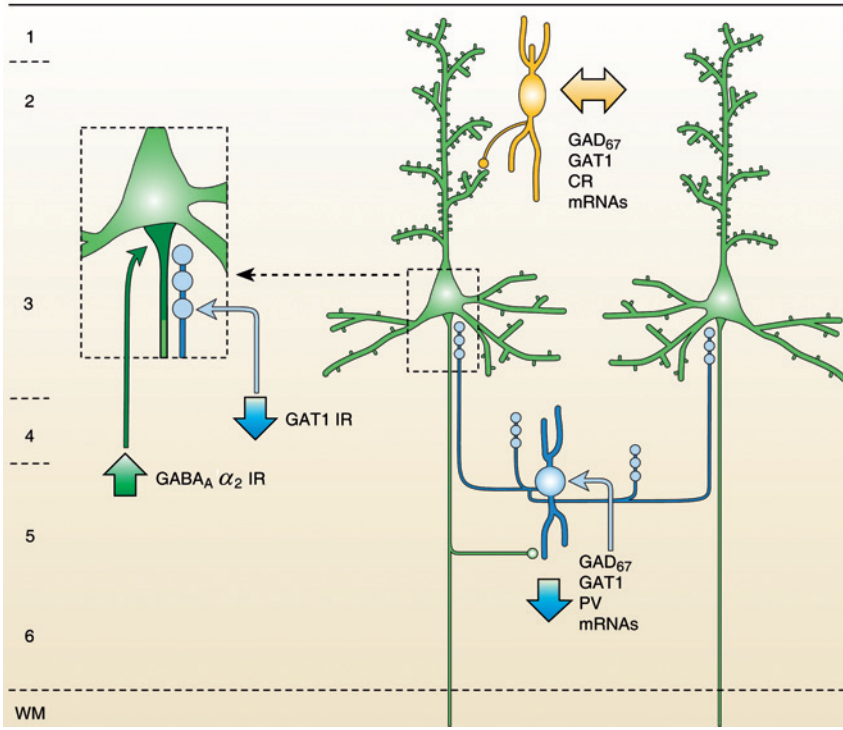

FIG. 3. Schematic summary of alterations in GABA circuitry in the DLPFC of subjects with schizophrenia. Reduced levels of gene expression in chandelier neurons (blue) are associated with a decrease in immunoreactivity (IR) for GAT1 in the axon cartridges of these neurons and an upregulation of $GABA_A$ receptor α_2 subunit immunoreactivity in the postsynaptic axon initial segment of pyramidal neurons (green). In contrast, gene expression does not appear to be altered in the calretinin-containing subclass of GABA neurons (yellow). Reprinted from Lewis *et al.* (2005).

the efficacy of GABA neurotransmission at pyramidal cell axon initial segments during the types of repetitive activity that are associated with working memory. However, it appears that in schizophrenia these compensatory mechanisms are not adequate to overcome the effects of decreased GABA synthesis in chandelier neurons.

Similar pre- and postsynaptic alterations might also be present in the inputs of PV-containing wide-arbor neurons to the perisomatic region of pyramidal neurons. For example, the density of PV-immunoreactive puncta, possibly the axon terminals of wide-arbor neurons (Erickson and Lewis, 2002), is reduced in the middle layers, but not in the superficial layers, of the DLPFC of subjects with schizophrenia (Lewis *et al.*, 2001), paralleling the laminar pattern of decreased PV

mRNA expression in schizophrenia (Hashimoto *et al.*, 2003). Furthermore, the increased density of $GABA_A$ receptors in the DLPFC of subjects with schizophrenia found in ligand-binding studies (Benes *et al.*, 1996; Hanada *et al.*, 1987) was most prominent at pyramidal neuron cell bodies (Benes *et al.*, 1996). Together, these data suggest that $GABA_A$ receptors located at the soma and axon initial segments of pyramidal neurons are locally upregulated in schizophrenia in response to a reduction in perisomatic inhibitory input from chandelier and wide-arbor neurons.

However, abnormalities in PV neurons alone may not completely account for the deficits in expression of GAD_{67} and GAT1 mRNAs since such changes were also observed in cortical layers 1 and 2, where relatively few PV-containing GABA neurons are located (Condé *et al.*, 1994) and where no changes in PV mRNA expression were found (Hashimoto *et al.*, 2003). Thus, other subpopulations of GABA neurons present in these layers, such as those that express the calcium-binding protein calbindin and/or the neuropeptides somatostatin or cholecystokinin, may also be altered in schizophrenia (Gabriel *et al.*, 1996; Virgo *et al.*, 1995). Indeed, we reported a marked decrease in mRNA levels for the somatostatin precursor protein in the DLPFC of subjects with schizophrenia (Hashimoto *et al.*, 2005a).

Are these abnormalities in GABA neurotransmission restricted to the DLPFC or representative of a disturbance distributed across other cortical regions that may contribute to other aspects of the clinical syndrome of schizophrenia? The anterior cingulate cortex, superior temporal gyrus, and hippocampal formation also appear to be sites of dysfunction in schizophrenia (Harrison and Lewis, 2003). Initial studies of the hippocampus reported a reduction in the density of nonpyramidal, putatative GABA neurons (Benes *et al.*, 1998) and an increase in $GABA_A$ receptor binding (Benes *et al.*, 1998) in schizophrenia. However, a study from the same research group did not detect a difference in the expression of either GAD_{67} or GAD_{65} mRNAs in the hippocampus of subjects with schizophrenia, although both transcripts were found to decrease in subjects with bipolar disorder (Heckers *et al.*, 2002). In the anterior cingulate cortex of subjects with schizophrenia, the densities of nonpyramidal neurons (Benes *et al.*, 1998) and of neurons immunoreactive for the calcium-binding protein calbindin (Cotter *et al.*, 2002) were reported to be reduced in layer 2, as was the density of GAD_{67} mRNA-positive neurons (Woo *et al.*, 2004). Within the superior temporal gyrus, GAD_{67} mRNA levels were found to be reduced (Impagnatiello *et al.*, 1998). In addition, the density of GAT1-immunoreactive axon cartridges was reduced in this region, although to a much lower extent than in the DLPFC of the same subjects with schizophrenia (Konopaske *et al.*, 2006). Thus, the available data suggest that alterations in GABA neurotransmission in schizophrenia may be a common feature across regions of the neocortex, but not of the hippocampus (Heckers *et al.*, 2002).

III. Potential Pathogenetic Mechanisms for Cell Type-Specific Alterations in GABA Neurons

Several different mechanisms have been suggested as the proximal cause of the alterations in PV-positive neurons in schizophrenia on the basis of correlated changes in individuals with the illness and evidence from animal models that these correlations represent cause and effect (Lewis *et al.*, 2005). Of these possibilities, alterations in N-methyl-D-aspartate (NMDA) receptor-mediated excitatory neurotransmission and deficits in neurotrophin signaling appear to have the strongest empirical basis.

A. Reduced Excitatory Drive via NMDA Receptors

The deficit in GAD_{67} mRNA expression has been suggested to represent an activity-dependent change in response to reduced activity of excitatory circuits in the DLPFC (Akbarian *et al.*, 1995; Jones, 1997). One source of such excitatory inputs is the mediodorsal nucleus of the thalamus, the principal source of thalamic projections to the DLPFC. Interestingly, initial studies reported that the number of neurons in this nucleus was reduced in subjects with schizophrenia (Byne *et al.*, 2002; Pakkenberg, 1990, 1992; Popken *et al.*, 2000; Young *et al.*, 2000); however, studies have failed to confirm these observations (Cullen *et al.*, 2003; Danos *et al.*, 2003; Dorph-Petersen *et al.*, 2004; Nielsen *et al.*, 2004; Young *et al.*, 2000). Furthermore, experimental reductions in neuron number in the mediodorsal thalamus of rodents did not produce alterations in the expression of GAD_{67} mRNA in the prefrontal cortex (Volk and Lewis, 2003). However, a range of other alterations, such as a decreased density of dendritic spines (a marker of excitatory synaptic inputs to pyramidal neurons) in the DLPFC of subjects with schizophrenia (Garey *et al.*, 1998; Glantz and Lewis, 2000), are consistent with reduced excitatory drive in DLPFC circuits, although the source of these altered inputs has yet to be determined.

One potential source of such altered inputs is the hippocampus. A variety of structural and functional abnormalities in the hippocampus have been observed in subjects with schizophrenia, and some of these appear to be correlated with alterations in the DLPFC (Bertolino *et al.*, 1996). To explore this association, investigators have employed a rodent model in which lesions of the ventral hippocampus are created neonatally (Lipska and Weinberger, 2000). In adulthood, these animals, in addition to mimicking a number of other phenotypic features of schizophrenia, show deficits in GAD_{67} expression in the prefrontal cortex (Lipska *et al.*, 2003). However, whether the deficits in GAD_{67} mRNA expression exhibit the cell type specificity, and are accompanied by the other changes in GABA markers present in schizophrenia, has not yet been examined.

These alterations in excitatory neurotransmission might differentially affect PV-, and not calretinin (CR)-containing GABA neurons because PV-containing

cells receive a larger complement of excitatory inputs (Lewis and Moghaddam, 2006). For example, in the rodent hippocampus, the total number of excitatory synapses onto PV-positive neurons is nearly an order of magnitude greater than the number onto CR-positive neurons (Gulyás *et al.*, 1999). Similarly, the density of asymmetric excitatory synapses on PV-positive dendrites in monkey DLPFC is significantly greater than on CR-positive dendrites (Melchitzky and Lewis, 2003). In addition, cell type differences have been reported for the NMDA receptor. For example, immunoreactivity for the NMDAR1 subunit was detected in the majority (50–90%) of PV-positive neurons, but in <10% of CR-positive neurons in monkey neocortex (Huntley *et al.*, 1994, 1997).

Convergent lines of evidence also indicate that PV-containing neurons are particularly sensitive to manipulation of excitatory signaling via NMDA receptors. First, administration of ketamine, an NMDA receptor antagonist, was associated with a decrease in the density of PV-immunoreactive neurons in the rodent hippocampus (Keilhoff *et al.*, 2004). Similarly, chronic exposure to PCP, another NMDA receptor antagonist, also resulted in decreased PV mRNA expression in the prefrontal cortex (Cochran *et al.*, 2003). Interestingly, in the latter study, the density of PV mRNA-positive neurons was unchanged following PCP, but the expression level of PV mRNA per neuron was decreased by 25%. These findings are strikingly similar to the pattern of PV mRNA expression changes observed in the DLPFC cortex of subjects with schizophrenia (Hashimoto *et al.*, 2003). Second, in cultures of mouse cortical neurons, ketamine induced a decrease in both PV and GAD_{67} immunoreactivity specifically in PV interneurons, an effect that appeared to be mediated by NR2A-, and not NR2B-containing NMDA receptors (Kinney *et al.*, 2006). Consistent with the cell-type selectivity of the effect, the ratio of NR2A/NR2B was observed to be fivefold higher in PV-positive neurons than in pyramidal cells. Third, in living slice preparations from mouse entorhinal cortex, both the genetically engineered reduction in lysophosphatidic acid-1 (LPA-1) receptor and the acute blockade of NMDA receptors produced a laminar-specific decrease in induced gamma oscillations (see below), and in the LPA-1-deficient animals, these physiological changes were associated with an ~40% laminar-specific reduction in the number of GABA- and PV-containing neurons, without a change in the number of CR-positive neurons (Cunningham *et al.*, 2006). Together, these findings suggest that the alterations in GABA neurotransmission selective for PV-containing neurons in schizophrenia might be a downstream consequence of impaired NMDA receptor-mediated glutamatergic inputs.

B. Reduced Neurotrophin Signaling

Signaling by the neurotrophin, brain-derived neurotrophic factor (BDNF), through its receptor tyrosine kinase (TrkB) promotes the development of GABA neurons and induces the expression of GABA-related proteins, including GAD_{67}

and GAT1 (Marty *et al.*, 2000; Yamada *et al.*, 2002). In addition, TrkB is predominantly expressed by PV-containing, and not by calretinin-containing, GABA neurons, suggesting cell type-specificity of these effects (Cellerino *et al.*, 1996). Indeed, in mice, genetically engineered to overexpress BDNF, the development of cortical GABA neurons was accelerated and accompanied by a precocious increase in the number of neurons containing PV (Huang *et al.*, 1999). Thus, reduced BDNF-TrkB signaling might be an "upstream" event contributing to the altered expression of GABA-related genes in the DLPFC of subjects with schizophrenia. Consistent with this hypothesis, the mRNA and protein levels for both BDNF and TrkB reduced in the DLPFC of subjects with schizophrenia (Hashimoto *et al.*, 2005b; Weickert *et al.*, 2003, 2005). In contrast, levels of the mRNA encoding the receptor tyrosine kinase for neurotrophin-3, TrkC, were unchanged (Hashimoto *et al.*, 2005b).

Comparisons with the results of other studies indicate that these reduced mRNA levels represent alterations in gene expression and not a loss of DLPFC neurons in schizophrenia. For example, total neuron number is not altered in the prefrontal cortex of subjects with schizophrenia (Thune *et al.*, 2001). In addition, the density of DLPFC pyramidal neurons has been reported to be modestly increased across cortical layers (Selemon *et al.*, 1995) or to be unchanged in layer 3 (Pierri *et al.*, 2003) in schizophrenia. Similarly, the density of all nonpyramidal neurons has been reported to be slightly increased (Selemon *et al.*, 1995) or unchanged (Akbarian *et al.*, 1995), and as noted above, the densities of PV-immunoreactive (Beasley *et al.*, 2002; Woo *et al.*, 1997) and PV mRNA-positive neurons (Hashimoto *et al.*, 2003) were not altered in the DLPFC of subjects with schizophrenia. Because BDNF mRNA is expressed by pyramidal neurons, and because TrkB mRNA is expressed in both pyramidal and PV-containing GABA neurons, the absence of a reduction in neuron number in both of these neuronal populations in schizophrenia indicates that the decrease in BDNF and TrkB mRNAs is due to a downregulation in the expression of the transcripts.

Consistent with the hypothesis that altered GABA-related gene expression is driven by reduced BDNF-TrkB signaling in schizophrenia subjects, the changes in TrkB and GAD_{67} mRNA expression levels were strongly correlated ($r = 0.74$, $p < 0.001$) in the same subjects, and a positive correlation between the changes in BDNF and GAD_{67} mRNA expression levels ($r = 0.52$, $p = 0.007$) was also observed. Interestingly, the correlation was significantly ($p = 0.043$) stronger between TrkB and GAD_{67} mRNAs than between BDNF and GAD_{67} mRNAs, suggesting that altered TrkB might be a pathogenetic mechanism driving the reduced GABA-related gene expression in schizophrenia.

Of course, such correlations in human studies do not demonstrate a cause and effect relationship. However, the idea that reduced signaling through TrkB receptors could be a primary determinant of cortical GABA-related gene expression changes in schizophrenia was supported by studies in TrkB hypomorphic

mice in which the insertion of floxed TrkB cDNA (*fBZ*) resulted in decreased TrkB expression (Xu *et al.*, 2000a). Compared to wild-type mice, TrkB mRNA expression levels in the prefrontal cortex were significantly decreased by 42% and 75% in mice with *fBZ*/+ and *fBZ*/*fBZ* genotypes, respectively, and in *fBZ*/*fBZ* mice, expression levels of GAD_{67} and PV mRNAs in the prefrontal cortex were significantly decreased by 25% and 40%, respectively (Hashimoto *et al.*, 2005b). In addition, in the *fBZ*/+ mice, the expression levels of GAD_{67} and PV mRNAs were intermediate between the wild-type control and *fBZ*/*fBZ* mice. Furthermore, the cellular pattern of reduced GAD_{67} mRNA expression in these mice precisely paralleled that seen in schizophrenia (Volk *et al.*, 2000). That is, the density of neurons with detectable levels of GAD_{67} mRNA was significantly reduced, but the level of GAD_{67} mRNA expression per neuron was unchanged (Hashimoto *et al.*, 2005b). Furthermore, consistent with the selective vulnerability of a GABA neuron subpopulation in schizophrenia, TrkB genotype had no effect on the expression of calretinin mRNA. Thus, the alterations in GABA-related gene expression in TrkB hypomorphic mice replicate those found in subjects with schizophrenia at both the tissue and cellular levels.

In contrast, although BDNF mRNA expression was decreased by 80% in the prefrontal cortex of mice with a neuron-specific inducible knockout of *bdnf* (Monteggia *et al.*, 2004), no alterations in the expression levels of GAD_{67} or PV mRNAs were present in these animals, whether the *bdnf* knockout was induced during embryogenesis or in adulthood (Hashimoto *et al.*, 2005b). Together, these findings suggest that changes in TrkB expression, and not in BDNF expression, regulate GABA-related gene expression in the prefrontal cortex.

Thus, these observations support the hypothesis that the deficit in expression of GAD_{67} mRNA in schizophrenia is the direct result of reduced TrkB expression in GABA neurons. However, this expression deficit may also be an indirect consequence of alterations in pyramidal neurons. For example, BDNF-TrkB signaling appears to promote somatodendritic development (McAllister *et al.*, 1995; Xu *et al.*, 2000b) and spine formation (Horch *et al.*, 1999) in pyramidal neurons, although spine density was not reduced in the prefrontal cortex of mice with the inducible knockout of *bdnf* (Hill *et al.*, 2005). Interestingly, in the DLPFC of subjects with schizophrenia, pyramidal neurons exhibit decreased somal size, dendritic length, and spine density (Garey *et al.*, 1998; Glantz and Lewis, 2000; Pierri *et al.*, 2001; Rajkowska *et al.*, 1998), consistent with an effect of reduced BDNF-TrkB signaling on pyramidal neurons in the illness. These findings, in concert with evidence that BDNF-TrkB signaling directly affects the efficacy of excitatory neurotransmission among pyramidal neurons (Kang and Schuman, 1995; Xu *et al.*, 2000a), suggest that reduced expression of TrkB in pyramidal neurons may both alter their morphology and cause a decrease in their activity. The resulting reduction in pyramidal neuron activity may lead to reduced gene expression in GABA neurons, especially those containing PV, which (in contrast

to calretinin-containing GABA neurons) receive direct excitatory inputs from neighboring pyramidal neurons (Melchitzky and Lewis, 2003).

IV. Connecting Alterations in PV-Positive Neurons to Working Memory Impairments: Decreased Gamma Band Synchrony in Schizophrenia

If reduced signaling via the TrkB receptor results in deficient chandelier cell-mediated inhibition of pyramidal neurons, how do such changes in GABA neurotransmission give rise to altered working memory? Interestingly, PV-containing inhibitory neurons are involved in the induction and maintenance of gamma oscillations in pyramidal neurons. In particular, networks of PV-containing, fast-spiking GABA neurons in the middle cortical layers, formed via both chemical and electrical synapses, give rise to oscillatory activity in the gamma band (30–80 Hz) range (Tamas *et al.*, 2000). The coordinated oscillatory context provided by these networks of inhibitory neurons is thought to create the discrete temporal structure necessary for ensembles of pyramidal neurons to perform specific functions, such as those involved in working memory.

Consistent with this interpretation, gamma band oscillations are induced and sustained in the DLPFC during the delay period of working memory tasks (Tallon-Baudry *et al.*, 1998). In addition, the amplitude, or power, of gamma oscillations in the DLPFC appears to increase in proportion to working memory load (Howard *et al.*, 2003). In the frontal cortex of subjects with schizophrenia, phase locking of gamma activity to the stimulus onset is impaired (Spencer *et al.*, 2003), and gamma band power in the DLPFC is reduced during the delay period of a working memory task (Cho *et al.*, 2004).

Thus, a deficit in pyramidal cell synchronization, resulting from impaired perisomatic inhibition via PV-containing GABA neurons, may contribute to the reported deficits in gamma oscillations, and consequently working memory dysfunction, in schizophrenia. Several features of PV-containing neurons, and of their alterations in schizophrenia, may explain how this occurs. First, the axonal arborizations of individual chandelier and wide-arbor neurons are highly divergent and target the axon initial segments and cell bodies, respectively, of a large number of pyramidal neurons (Peters, 1984), enabling them to regulate the firing of local groups of pyramidal neurons (Cobb *et al.*, 1995). Second, in the monkey DLPFC, PV-containing GABA neurons and pyramidal cells share certain excitatory inputs in common, including projections from neighboring pyramidal neurons and from the mediodorsal thalamus (Melchitzky and Lewis, 2003; Melchitzky *et al.*, 1999, 2001). Thus, excitatory input from these sources stimulates both PV-containing and pyramidal neurons simultaneously, resulting in a secondary, temporally delayed

perisomatic inhibitory input to pyramidal neurons. This disynaptic inhibitory input appears to limit the window of time, and thereby increases the temporal precision, for the summation of excitatory inputs needed to evoke pyramidal neuron firing (Pouille and Scanziani, 2001). Consequently, a deficiency in chandelier cell and/or wide-arbor neuron perisomatic input to pyramidal neurons would be expected to reduce the magnitude of pyramidal cell synchrony, and thus of gamma band power, in the DLPFC.

V. Treatment Implications

How do these findings inform our understanding of novel targets for pharmacological intervention in schizophrenia? Drugs with selective agonist activity at $GABA_A$ receptors containing the α_2 subunit may provide an effective approach to enhance chandelier neuron inhibition of DLPFC pyramidal neurons in schizophrenia by increasing the synchronization of pyramidal cell firing at gamma frequencies and consequently improving working memory function (Lewis *et al.*, 2004; Volk and Lewis, 2005). The α_2 subunit of the $GABA_A$ receptor represents a highly selective target for enhancing inhibition at the axon initial segment of pyramidal neurons because it is predominantly restricted to this location (Nusser *et al.*, 1996).

Drugs that directly activate α_2-containing $GABA_A$ receptors independent of the presence of GABA, or that generally increase the firing rate of chandelier cells, may actually disrupt the timing of disynaptic inhibition of pyramidal neurons. Furthermore, agents that nonselectively inhibit GABA reuptake may act too broadly at other GABA synaptic sites that are not altered in schizophrenia. In contrast, drugs that enhance the postsynaptic response to the release of GABA from chandelier cell axons, such as a $GABA_A$ α_2-selective benzodiazepine, would increase the frequency of opening of chloride ion channels in the presence of GABA. Currently available benzodiazepines are not selective for $GABA_A$ receptors containing the α_2 subunit, and might cause a generalized and nonspecific increase in cortical inhibition. Thus, treatment with an α_2-selective benzodiazepine would be predicted to augment the postsynaptic inhibitory response at pyramidal neuron axon initial segments in a manner that incorporates the critical timing of chandelier neuron firing essential for synchronizing pyramidal neuron activity. Furthermore, since the anxiolytic effects of benzodiazepines appear to be mediated by $GABA_A$ receptors that contain the α_2 subunit (Löw *et al.*, 2000), α_2-specific agents may both improve cognitive function and reduce the stress responses that have been linked to the exacerbations of psychotic symptoms in schizophrenia (Carpenter *et al.*, 1999).

Acknowledgments

Work by the authors cited in this manuscript was supported by National Institutes of Health grants MH 045156, MH 051234, and MH 043784, and by a NARSAD Young Investigator Award (TH).

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ALTERATIONS OF SEROTONIN TRANSMISSION IN SCHIZOPHRENIA

Anissa Abi-Dargham

Department of Psychiatry, Columbia College of Physicians and Surgeons
New York State Psychiatric Institute, Columbia University, New York 10032, USA

A role for serotonin alterations in the pathophysiology of schizophrenia has long been suspected because of the psychotogenic effects of serotonergic agonists and the therapeutic effects of 5-HT_2 antagonism. This chapter is a review of the evidence derived from pharmacological studies, postmortem, and imaging studies that have assessed the role of serotonin transmission in schizophrenia. While a clear picture of specific serotonergic alterations in schizophrenia has not emerged despite much research, this review reinforces a modulatory role of serotonergic agents on dopamine transmission in schizophrenia, which may contribute to the therapeutic effects of atypical antipsychotics.

I. Introduction

A dysfunction of 5-hydroxytryptamine (5-HT) function in schizophrenia was first postulated because of the structural similarity between 5-HT and the hallucinogenic drug lysergic acid diethylamide (LSD; Gaddum, 1954;

INTERNATIONAL REVIEW OF NEUROBIOLOGY, VOL. 78
DOI: 10.1016/S0074-7742(06)78005-9

0074-7742/07 $35.00

Wooley and Shaw, 1954) and renewed after the introduction of clozapine in the United States, a drug with negligible liability for extrapyramidal side effects (EPS) and superior antipsychotic properties compared to typical antipsychotics (Kane *et al.*, 1988). The superior efficacy of clozapine has been attributed to its relatively potent 5-HT_2 receptor antagonism (Meltzer, 1991), prompting the development of pure 5-HT_2 antagonists or "balanced" 5-HT_2-D2 antagonists as potential antipsychotics. So far, available data indicates acceptable antipsychotic efficacy for combined 5-HT_2-D2 antagonists, but not for pure 5-HT_2 antagonists. At the same time, we have learned more about the potential role of other serotonergic receptors in the action of atypical antipsychotics. In addition, postmortem studies, cerebrospinal fluid (CSF), clinical challenge, and imaging studies of serotonergic receptors and transporters performed over the last decade have suggested a serotonergic dysfunction in the brains of patients with schizophrenia. In this chapter, we will first review the evidence for alterations of serotonin transmission in schizophrenia and its implications for the therapeutic effects of antipsychotics. The putative role of 5-HT transmission in schizophrenia will then be discussed in the context of all the data reviewed, the relevant dopaminergic and serotonergic interactions, and the recent advances in the conceptualization of the dopamine (DA) hypothesis of schizophrenia.

II. Alteration of 5-HT Receptors in Schizophrenia

The involvement of alteration of 5-HT transmission in the pathophysiology of schizophrenia is supported by numerous postmortem studies, which have been reviewed elsewhere (Abi-Dargham *et al.*, 1997; Breier, 1995; Lieberman *et al.*, 1998; Meltzer *et al.*, 1999). The most consistent abnormalities of 5-HT markers in schizophrenia are a reduction in cortical 5-HT transporters density and an increase in cortical 5-HT_{1A} receptor binding. A decrease in 5-HT_{2A} density has also been frequently noted, but this observation might be secondary to previous neuroleptic exposure (Table I).

A. 5-HT Transporters

5-HT transporters are located on presynaptic serotonergic terminals and are believed to provide an index of serotonergic innervation. Three studies reported decreased density of 5-HT transporters in the frontal cortex of patients with schizophrenia. Laruelle *et al.* (1993) reported decreased density of 5-HT transporters, labeled with [^{3}H]paroxetine, in the frontal cortex of schizophrenic patients, as compared to controls, while no changes were observed in the

TABLE I

ALTERATIONS IN 5-HT RECEPTORS IN SCHIZOPHRENIA: POSTMORTEM STUDIES

References	Site	Ligand	*B*max	K_D	Brain region
Ohuaha *et al.* (1993)	5-HT uptake site	[3H]citalopram	Decrease	No change	Frt Ctx
Laruelle *et al.* (1993)		[3H]citalopram	Decrease	No change	Frt Ctx
Joyce *et al.* (1993)		[3H]cyanoimipramine	Decrease	No change	Frt Ctx
Dean *et al.* (1995)		[3H]citalopram	No change	Increase	Hippocampus
Hashimoto *et al.* (1991)	5-HT_{1A}	[3H]8-OH-DPAT	Increase		Frt Ctx, BA 10 Temp Ctx
Joyce *et al.* (1993)		[3H]8-OH-DPAT	Nonsignificant increase		Frt Ctx BA 9, Cing Ctx Motor Ctx, Hippoc.
Simpson *et al.* (1996)		[3H]8-OH-DPAT	Increase		Frt Ctx BA 12 and 11
Sumiyoshi *et al.* (1996)		[3H]8-OH-DPAT	Increase		PFC
Burnet *et al.* (1996b)		[3H]8-OH-DPAT	Increase		Frt Ctx BA 46
Burnet *et al.* (1997)		[3H]8-OH-DPAT	Increase		Frt Ctx BA 46
Gurevich *et al.* (1997)		[3H]WAY-100635	Increase		Frt Ctx BA 9, 44, 6, Cing Ctx
Dean *et al.* (1999)		[3H]8-OH-DPAT	No change		Frt Ctx BA 9, 8, 10
Bennett *et al.* (1979)	5-HT_2	[3H]LSD	Decrease	No change	Frt Ctx
Whitaker *et al.* (1981)		[3H]LSD	No change	No change	Frt Ctx
Mita *et al.* (1986)		[3H]ketanserin	Decrease	No change	Frt Ctx
Arora and Meltzer (1991)		[3H]spiperone	Decrease	No change	Frt Ctx
Reynolds *et al.* (1983)		[3H]ketanserin	No change	No change	Frt Ctx
Laruelle *et al.* (1993)		[3H]ketanserin	No change	No change	Frt Ctx
Joyce *et al.* (1993)		[3H]ketanserin	No change	No change	Frt Ctx
Dean *et al.* (1999)		[3H]ketanserin	Decrease		Frt Ctx BA 9
Abi-Dargham *et al.* (1993)	5-HT_3	[3H]LY278584	No change	No change	Amygdala
Dean *et al.* (1999)	5-HT_4	3H]GR113808	No change		Frt Ctx BA 9, 8, 10

occipital cortex of the same subjects (Laruelle *et al.*, 1993). Joyce *et al.* (1993) reported decreased 5-HT transporter density, labeled with [^{3}H]cyanoimipramine, in the frontal and cingulate cortices in patients with schizophrenia, while no changes were observed in the motor cortex, temporal cortex, and hippocampus. In the same schizophrenic patients, increased 5-HT transporter density was observed in the striatum. Ohuoha *et al.* (1993) reported decreased density of 5-HT transporters, labeled with [^{3}H]citalopram in the frontal cortex of schizophrenic patients as compared to controls. Dean *et al.* (1995) reported decreased affinity of the transporters in the hippocampus but did not replicate the findings of decreased 5-HT transporters in the frontal cortex in schizophrenia, possibly due to methodological differences, as they examined a different area of the frontal cortex than did the other investigators. Similarly negative findings were reported by Gurevich *et al.* (1997).

Brain-imaging studies provide the opportunity to study well-characterized and medication free patients. However, the development of radiotracers for *in vivo* imaging of serotonin transporter (SERT) has been difficult. [^{11}C] 3-Amino-4-(2-dimethyl-aminomethyl-phenylsulfanyl)-benzonitrile (DASB) was successfully produced and evaluated in humans (Ginovart *et al.*, 2001; Houle *et al.*, 2000; Meyer *et al.*, 2001). [^{11}C]DASB provides higher specific to nonspecific binding ratios than previous tracers such as [11C]McN 5652, and thus an enhanced reliability in the assessment of SERT density (Frankle *et al.*, 2004; Huang *et al.*, 2002; Szabo *et al.*, 2002).

We used [^{11}C]DASB to compare SERT availability between medication-free subjects with schizophrenia and matched controls. Brain regions included in this analysis were those where the concentration of SERT is high enough for [^{11}C]DASB to provide reliable quantification of SERT availability. There were no significant group differences in SERT availability in any brain region. This study failed to support the postmortem findings of decreased SERT affinity in the hippocampus in schizophrenia (Dean *et al.*, 1995; Naylor *et al.*, 1996) as well as the decrease in SERT in the cingulate cortex observed in one postmortem study (Joyce *et al.*, 1993). In the midbrain, this study agreed with the negative results of the previous imaging study with [^{123}I]β-CIT (Laruelle *et al.*, 2000).

In addition, the level of SERT binding was not significantly related to the severity of positive, negative, or depressive symptoms.

Studies in larger samples with radiotracers which allow quantification of cortical 5-HT transporters may be needed to provide a more definitive picture of serotonin innervation in brains of patients with schizophrenia.

B. 5-HT$_{1A}$ Receptors

As noted by Joyce, "Postmortem studies of the concentration of 5-HT$_{1A}$ receptors in schizophrenic patients represent a rare consensus in schizophrenia: all studies published to date found an elevation of this receptor subtype in

schizophrenic patients" (Gurevich and Joyce, 1997). With the exception of one negative study (Dean *et al.*, 1999), seven out of eight studies reported elevations of 5-HT_{1A} receptors in frontal cortex of patients with schizophrenia (Burnet *et al.*, 1996b, 1997; Gurevich and Joyce, 1997; Hashimoto *et al.*, 1991; Joyce *et al.*, 1993; Simpson *et al.*, 1996; Sumiyoshi *et al.*, 1996). All studies were performed with the 5-HT_{1A} receptor agonist [^{3}H]8-hydroxy-2-[di-n-propyl-amino]tetralin ([^{3}H]8-OH-DPAT), except for the study of Burnet *et al.* (1997), which used [3H] WAY-100635. Two studies were performed with homogenate binding and five with autoradiography. All studies included samples from the Prefrontal Cortex (PFC): a significant increase has been reported in all prefrontal Brodmann areas (BA) studied, including the dorso-lateral-prefrontol cortex (DLPFC) (areas 8, 9, 45, and 46), the frontal pole (BA 10), the orbitofrontal cortex (OFC) (BA 11 and 12), and some premotor areas (BA 44, 6). The effect size (difference in the means divided by SD) varied considerably, from small (0.24) to large (1.13), with an average effect size of 0.85 ± 0.34. A similar effect size was obtained when the average was weighted by the number of cases included per study. Combined, these studies reached a significant level of $p = 0.0006$. Other regions were evaluated: in the cingulate, two studies reported an increase and two found no change. An increase was also reported in the temporal cortex, motor cortex, and hippocampus, but none of these findings were confirmed by the other studies.

The finding of increased 5-HT_{1A} receptors was reported in patients on and off drugs at time of death. Moreover, subchronic (21 days) treatment with haloperidol or clozapine does not affect the density of prefrontal 5-HT_{1A} receptors (Burnet *et al.*, 1996a; Shapiro *et al.*, 1995; Stockmeier *et al.*, 1996). However, as most patients received antipsychotics and other psychotropic medications for years, the possibility that this increase may be a long-term effect of treatment cannot be excluded. An increase in 5-HT_{1A} receptors in the OFC (but not the DLPFC) has also been reported in suicide victims (Arango *et al.*, 1995), raising questions about the disease specificity of this alteration in patients with schizophrenia. However, four studies failed to detect abnormalities in [3H]8-OH-DPAT binding in the frontal cortex of suicide victims (Arranz *et al.*, 1994; Dillon *et al.*, 1991; Lowther *et al.*, 1997; Matsubara *et al.*, 1991). In the PFC, 5-HT_{1A} receptors are concentrated in layers I-II, with lower densities in layers III-IV (Dillon *et al.*, 1991; Hoyer *et al.*, 1986a; Joyce *et al.*, 1993; Lidow *et al.*, 1989). At the ultrastructural level, prefrontal neuronal 5-HT_{1A} receptors are mostly present on the axon hillock of pyramidal cells (Azmitia *et al.*, 1996; Francis *et al.*, 1992). Striatal lesions which induce a degeneration of corticostriatal projections decrease 5-HT_{1A} receptors in the deep cortical layers, indicating that 5-HT_{1A} receptors are located on pyramidal cells projecting to the striatum (Francis *et al.*, 1992). In primate pyramidal cortical neurons, 5-HT_{1A} receptors are observed in high levels in the initial segment of the axons (axon hillock; Azmitia *et al.*, 1996). This localization is consistent with inhibition of action potential by 5-HT_{1A}

agonists (Azmitia *et al.*, 1996). Given the postmortem and clinical suggestion of reduced 5-HT innervation in the PFC in schizophrenia, one could propose that the increase in 5-HT_{1A} receptors observed in this region may be due to a functional upregulation of these receptors. This hypothesis is not supported by a majority of studies in rodents, which failed to observe an upregulation of 5-HT_{1A} receptors following 5.7-dihydroxytryptamine lesions of the 5-HT system (Hensler *et al.*, 1991; Kia *et al.*, 1996; Lawrence *et al.*, 1993; Pranzatelli *et al.*, 1994). Nevertheless, if the alteration of the 5-HT system in the PFC in schizophrenia is neurodevelopmental, the relevance of lesions in adult rodents is limited. A neurodevelopmental alteration is suggested by the study of Slater *et al.* (1998) showing a lack of regression of vermal 5-HT_{1A} receptors in cerebellum of patients with schizophrenia compared to controls.

Unfortunately this consistent data from postmortem studies received little support from the imaging studies undertaken to better explore the clinical relevance of these findings.

Three studies examined 5-HT_{1A} receptor levels in schizophrenia relative to controls *in vivo* using [11C]WAY 100635 positron emission tomography (PET). In the first study, Tauscher *et al.* (2002) reported an overall increase in [11C]WAY 100635 binding across nine regions in subjects with schizophrenia compared to controls, with post hoc analysis revealing significant difference in the medial temporal cortex. The second PET study to explore this issue found a reduction in 5-HT_{1A}-binding parameters in the amygdala (Yasuno *et al.*, 2004).

In the third study, we used [11C]WAY 100635 to compare 5-HT_{1A} availability between medication free subjects with schizophrenia and matched controls. We investigated the same brain regions where differences in the density of 5-HT_{1A} receptors in schizophrenia have been described as well as additional regions in which the concentration of 5-HT_{1A} is high enough for [11C]WAY 100635 to provide reliable quantification of 5-HT_{1A} availability. These regions included four prefrontal cortical regions (dorsolateral, medial, orbital, and subgenual), the parietal, temporal, and occipital cortices, the anterior cingulate cortex, insular cortex, the medial temporal lobe (amygdala, entorhinal cortex, hippocampus, and parahippocampal gyrus), and the dorsal raphe nucleus. We found no significant group differences in 5-HT_{1A} availability in any brain region. Within the patient group, the level of 5-HT_{1A} binding was not significantly related to the severity of positive, negative, or depressive symptoms. The explanation for the difference in findings among all three studies, reporting increase, decrease, and no change, in our case, is not clear. The sample size in the present study was slightly larger than either of the two prior studies. There were fewer drug-naive subjects in our sample compared to the study of Tauscher *et al.* (2002). However, we did not detect a difference in [11C]WAY 100635 binding when the data from these individuals was analyzed separately. Slight differences in methodology exist across the studies,

including a longer scan time in the current study (110 min vs 60 min; Tauscher *et al.*, 2002, and 90 min; Yasuno *et al.*, 2004) and different data modeling strategies (kinetic modeling with arterial input in the current study compared with a reference region approach in the other two studies). These technical differences would be expected to affect the schizophrenic and control groups equally within each study, and therefore one would expect similar results across studies. In fact, when we analyzed our data using a reference region approach we found no differences between the groups.

Although differences exist between these three PET studies, none of the studies confirmed the findings reported in the postmortem literature exploring 5-HT_{1A} density in schizophrenic subjects. The postmortem studies report an increase in 5-HT_{1A} density in subjects with schizophrenia when compared to controls. This increase is observed in the dorsolateral prefrontal cortex in most studies (Burnet *et al.*, 1996b, 1997; Gurevich and Joyce, 1997; Hashimoto *et al.*, 1991; Simpson *et al.*, 1996; Sumiyoshi *et al.*, 1996), as well as in the anterior cingulate (Burnet *et al.*, 1996b; Gurevich and Joyce, 1997; Joyce *et al.*, 1993) and motor cortex (Gurevich and Joyce, 1997; Joyce *et al.*, 1993) in others. The reason for this lack of consistency in findings may relate to the resolution of these very different techniques. Some postmortem studies show more pronounced increase in 5-HT_{1A} density within superficial cortical layers (Gurevich and Joyce, 1997), whereas others show no difference while exploring specific cellular locations within the prefrontal cortex such as the axon initial segment (Cruz *et al.*, 2004). Using PET, with the currently available technology, it is not possible to explore layer specific differences in receptor density within the cortex, limiting the resolution of these types of studies to relatively large cortical regions.

C. 5-HT_2 Receptors

Studies of 5-HT_2 receptors in the frontal cortex of patients with schizophrenia have generated conflicting results. Early studies were performed with [^{3}H]LSD, a ligand which labels both 5-HT_1 and 5-HT_2 families (Peroutka and Snyder, 1979). Bennett *et al.* (1979) reported decreased [^{3}H]LSD binding in the frontal cortex of patients with schizophrenia as compared to controls. Because [^{3}H]5-HT binding was not reduced in these samples, the decreased [3H]LSD binding was attributed to the 5-HT_{2A} sites, which display relatively low affinity for 5-HT. A second study performed with [^{3}H]LSD showed no differences in the frontal cortex between schizophrenic and control samples (Whitaker *et al.*, 1981).

The more selective 5-HT_2 ligands [^{3}H]ketanserin and [^{3}H]spiperone, were used in five studies to evaluate frontal density of 5-HT_2 receptors. Since both ligands are relatively more selective for 5-HT_{2A} than 5-HT_{2C} (Choudhary *et al.*, 1992; Leysen, 1990), these studies can be viewed as measuring the 5-HT_{2A} rather

than the 5-HT_{2C}. Three studies demonstrated a significant decrease in 5-HT_2 density in the frontal cortex of schizophrenic patients (Arora and Meltzer, 1991; Dean *et al.*, 1999; Mita *et al.*, 1986) while no changes were reported in the other three studies (Joyce *et al.*, 1993; Laruelle *et al.*, 1993; Reynolds *et al.*, 1983). Given that 5-HT_2 antagonists downregulate 5-HT_2 receptors (Andree *et al.*, 1986; Helmeste and Tang, 1983; Leysen *et al.*, 1987) and that most antipsychotic drugs display 5-HT_2 antagonism (Leysen *et al.*, 1978, 1982; Wander *et al.*, 1987), these differences may reflect differences in the antemortem medication. Supporting this interpretation, a PET study with [^{18}F]setoperone failed to detect any significant changes in 5-HT_2 density in drug-naive patients with schizophrenia (Lewis *et al.*, 1997). Alternatively, these differences in the density of 5-HT_2 receptors in the frontal cortex in schizophrenia may be related to the heterogeneity of the disease. Laruelle *et al.* (1993) observed that, while schizophrenic patients who committed suicide had 5-HT_2 levels comparable to controls, schizophrenic patients who died from natural causes had significantly lower 5-HT_2 levels than controls. Interestingly, none of the patients in the series of Mita *et al.* (1986) and Arora *et al.* (1991) committed suicide. Thus, three studies suggest decreased frontal 5-HT_2 density in nonsuicide schizophrenic patients. The cause of death was not reported by Reynolds *et al.* and the series of Joyce *et al.* included both suicide and nonsuicide victims. The significance of this finding is unclear. Suicide per se has been associated with increased frontal 5-HT_2 receptor density in some (Arango *et al.*, 1990; Hrdina *et al.*, 1993; Mann *et al.*, 1986; Stanley and Mann, 1983) but not all (Cheetham *et al.*, 1988; Gross-Isseroff *et al.*, 1990; Laruelle *et al.*, 1993; Lowther *et al.*, 1994; Owen *et al.*, 1983, 1986) postmortem studies. Therefore, this factor has to be controlled for in studies of 5-HT_2 density in schizophrenia as it may account for some of the discrepancies in the findings. Decreased 5-HT_2 density may be associated with predominantly negative symptoms and a lower incidence of suicide.

Three PET studies in drug-naive or drug-free patients with schizophrenia reported normal cortical 5-HT_{2A} receptor binding (Lewis *et al.*, 1999; Okubo *et al.*, 2000; Trichard *et al.*, 1998), while one study reported a significant decrease in PFC 5-HT_{2A} binding in a small group ($n = 6$) of drug-naive schizophrenic patients (Ngan *et al.*, 2000).

D. Other Receptors

No change in the density of 5-HT_3 receptors was observed in the amygdala of patients with schizophrenia (Abi-Dargham *et al.*, 1993). A study found no change in the density of 5-HT_4 receptors in frontal cortex of patients with schizophrenia (Dean *et al.*, 1999).

III. Pharmacological Manipulation of 5-HT Transmission in Schizophrenia

In addition to the direct evidence reviewed above that 5-HT transmission might be affected in schizophrenia, pharmacological interventions modifying 5-HT transmission provided data implicating 5-HT transmission in the mediation of schizophrenia symptomatology. This evidence is specially compelling regarding interventions modifying 5-HT_{2A} receptor function.

A. 5-HT Precursors

The amino acid L-tryptophan is the dietary precursor of 5-HT. Administration of large doses of L-tryptophan increases the synthesis of 5-HT in the brain (Wurtman *et al.*, 1981). During the 1960s and 1970s, numerous studies examined the effects of 5-HT precursors on the clinical symptoms of schizophrenia. Lauer *et al.* (1958) and Pollin *et al.* (1961) administered tryptophan with iproniazid to schizophrenic patients and reported mood elevation, increased involvement, and motor activity. Given the concomitant use of Monoamine oxidase inhibitors (MAOI), these data are difficult to interpret. Bowers (1970) reported mild improvement in Brief Psychiatric Rating Scale (BPRS) in schizophrenic patients treated with L-tryptophan at doses of 2–4 g/day in combination with vitamin B6. Gillin *et al.* (1976) observed that tryptophan administration (20 g/day) had no effect in schizophrenia. Chouinard *et al.* (1978) tested the clinical efficacy of tryptophan (2–6 g/day) and benserazide coadministration against chlorpromazine, and concluded that the antipsychotic action of the tryptophan-benserazide combination was inferior to that of chlorpromazine. Morand *et al.* (1983) described a decrease in aggressivity in schizophrenic patients treated by tryptophan (4 g/day). In summary, tryptophan administration may produce a limited improvement in negative symptoms. Of interest is the fact that worsening of psychosis was not reported. Similar results were reported during the administration of the immediate 5-HT precursor, L-5-hydroxytryptophan, although some patients presented exacerbation of psychotic symptoms, maybe related to the fact that L-5-hydroxytryptamine administration increases also catecholaminergic function (Bigelow *et al.*, 1979; van Praag *et al.*, 1987; Wyatt *et al.*, 1972).

B. 5-HT Depleting Agents

Two studies with limited number of patients investigated the effects of the tryptophan hydroxylase inhibitor, *p*-chlorophenylalanine (pCPA). Casachia *et al.* (1975) reported improvement in three out of four acute schizophrenics during pCPA treatment (1250 mg/day), while DeLisi *et al.* (1982) reported no significant

changes in chronic schizophrenic patients ($n = 7$, 3000 mg/day). Fenfluramine, a halogenated amphetamine derivative, is believed to cause depletion of the serotonergic system when administered chronically. Shore *et al.* (1985) and Stahl *et al.* (1985) failed to show significant changes during fenfluramine treatment in placebo-controlled studies (8–12 weeks). Soper *et al.* (1990) demonstrated that fenfluramine treatment produced worsening of communication competence and thought disorder in treatment-resistant schizophrenic patients. In summary, these studies suggest that 5-HT depleting agents are not useful in the treatment of schizophrenia, and may even further impair cognitive functioning.

C. 5-HT_{2A} Agonism: LSD and "Model" Psychosis

The observation of an LSD-induced psychosis in healthy subjects was the first indication of a potential relationship between serotonin function and schizophrenia. The early reports in the 1950s emphasized the clinical similarities between LSD-induced psychosis and schizophrenia (Rinkel *et al.*, 1955). These were followed by numerous studies which examined carefully the differences such as the prevalence of visual as opposed to auditory hallucinations, the absence of thought disorder, the preservation of affect and insight (Hollister, 1962). However, these differences were lessened when the comparison involved early as opposed to chronic schizophrenics (Freedman and Chapman, 1973) and when cross-cultural differences in schizophrenic symptomatology were examined (Murphy *et al.*, 1963). One study (Langs and Barr, 1968) attempted to compare LSD effects to the different subtypes of schizophrenia and found similarities between the drug group and the paranoid but not the undifferentiated patients. Interestingly enough, the authors described a higher rate of overlap of symptoms for those drug subjects with poorly integrated premorbid personality. Overall, LSD-induced psychosis seemed to be a potential model for some (i.e., hallucinations and paranoid delusions), but not all aspects of schizophrenia (such as disorganization and negative symptoms; Fishman, 1983). Administration of mescaline (3,4,5-trimethoxyphenethylamine), a phenethylamine hallucinogen, to healthy volunteers resulted, similarly, in symptoms of dissolution of ego boundaries, visual hallucinations, "oceanic boundlessness," and passivity experiences (Hermle *et al.*, 1992). Similar findings were described in humans with psilocybin (Vollenweider *et al.*, 1998). Disturbances in performance on neuropsychological tasks and alterations of cerebral blood flow measured with single photon emission tomography and ^{99}Tm-HMPAO have also been described.

1. *Neuropharmacological Effects of Hallucinogens*

The first observation of an effect of LSD on serotonergic transmission was made in 1961 by Freedman (1961). Since, with the discovery of more than

15 subtypes of 5-HT receptors, much more is known about the effects of LSD on central serotonergic receptors. LSD inhibits serotonergic cells in raphe nuclei through a direct agonism on the presynaptic 5-HT_{1A} site, thus reducing the firing of these neurons and the release of serotonin. It also acts as a weak agonist on the postsynaptic 5-HT_{1A} site. LSD has high affinity for all other 5-HT_1 subtypes and for 5-$HT_{5A,5B}$ (Matthes *et al.*, 1993), 5-HT_6 (Shen *et al.*, 1993), and 5-HT_7 receptors. However, the hallucinogenic effect of LSD has been linked to its affinity for the 5-HT_2 receptor, as this property is shared by substituted phenethylamine hallucinogens, such as mescaline, DOI (1-(2,5-dimethoxy-4-iodophenyl-2-amino-propane hydrochloride), DOB (4-bromo-2,5-dimethoxyphenylisopropylamine), and DOM (2,5-dimethoxy-4-methylamphetamine) (Aghajanian, 1994), and other indoleamine hallucinogens such as DMT (*N,N*-dimethyltryptamine) and psilocybin. Phenethylamine hallucinogens are in general more selective for the 5-HT_2 receptor than LSD. A strong correlation was described between effective doses of indoleamines (LSD) and phenethylamine hallucinogens and their respective potency at the 5-HT_2 receptor (Glennon and Titeler, 1984; Titeler *et al.*, 1988), suggesting that 5-HT_2 receptors mediate the hallucinogenic effects of these drugs. Most data indicates a specific 5-HT_{2A} mechanism, although a 5-HT_{2C} effect cannot be ruled out. LSD has been reported to be an antagonist at the 5-HT_2 site by some investigators, and a full or partial agonist (Glennon, 1990) by others. However, data demonstrates clearly a partial agonist effect of LSD and DOI on the 5-HT_{2A} receptors in the piriform cortex in the rat (Marek and Aghajanian, 1996). This partial agonism effect may explain why LSD can appear as an antagonist, since it can decrease the effect of the agonist, when coadministered at high doses. Its dual effect on 5-HT_2 (stimulatory) and 5-HT_{1A} (inhibitory) can also explain how it may appear as an antagonist, since it can modulate its own effect. Psilocybin's psychotomimetic effects in humans were blocked by ketanserin, a pure 5-HT_{2A} antagonist, and risperidone (Vollenweider, 1998). As psilocybin is a potent 5-HT_{2A} and weak 5-HT_{1A} agonist, this study demonstrates, in humans, that 5-HT_{2A} activation is psychotogenic. Another study by the same group showed that this psychosis may be, at least partly, mediated by an increased release of DA, as evidenced by a 20% decrease of [^{11}C]racploride binding after psilocybin administration in the striatum of human subjects (Vollenweider *et al.*, 1999).

2. *Anatomical Substrates of Hallucinogens*

5-HT_{2A} receptors are present in high concentration in the olfactory bulb, hippocampus, frontal cortex, piriform, and entorhinal cortices, while 5-HT_{2C} receptors are present in highest density in choroid plexus, anterior olfactory nucleus, piriform and entorhinal cortices, striatum, amygdala, and Substantia Nigra (SN) (Hoyer *et al.*, 1986a,b; Martin and Humphrey, 1994). Hallucinogens have been shown to interact with 5-HT_2 receptors in the locus coeruleus (LC) and the cortex in rats. In the

LC, their effects were reversed by 5-HT_2 antagonists (Rasmussen and Aghajanian, 1986) and by various antipsychotics (Rasmussen and Aghajanian, 1988). The reversal of effect was correlated with the 5-HT_2 binding affinity of the antipsychotic medications. In the piriform cortex in the rat it has been shown that serotonin induces activation of GABAergic interneurons through the 5-HT_{2A} receptor resulting in an enhancement of spontaneous inhibitory postsynaptic potentials in the pyramidal cells (IPSPs) (Gellman and Aghajanian, 1993; Marek and Aghajanian, 1994; Sheldon and Aghajanian, 1991). Aghajanian and Marek (1999) have shown that 5-HT, through 5-HT_{2A} receptors, enhances spontaneous excitatory postsynaptic potentials (EPSPs) in pyramidal cells of layer V of the neocortex, through a focal action on apical dendrites, the main targets for excitatory corticocortical and thalamocortical inputs. This activation leads to an increase in asynchronous release of glutamate by pyramidal cells. This suggests a facilitation of glutamatergic transmission in the cortex via 5-HT_{2A} agonism, and may be consistent with the data of Farber *et al.* (1998) showing that 5-HT_{2A} agonism can prevent the vacuolization related to NMDA neurotoxicity in rodent brain. However, this increase in glutamate release can lead to an alteration in corticocortical and corticosubcortical transmission.

In summary, these studies overall suggest a strong relationship between 5-HT_{2A} stimulation and hallucinogen-induced psychosis. More similarities have been described between hallucinogen-induced psychosis and positive symptoms of schizophrenia as opposed to negative symptoms. Thus, one can conclude that alterations in 5-HT_{2A} function may mediate positive symptoms of schizophrenia, possibly by affecting directly or indirectly other transmitter systems such as DA and glutamate. This is consistent with the therapeutic efficacy of the new atypical neuroleptics known to have a strong 5-HT_2 antagonism.

D. 5-HT_{2A} Antagonism, Clozapine, and Atypicality

Almost all antipsychotic drugs have appreciable affinity for the 5-HT_2 receptors. 5-HT_2 receptors were initially termed "serotonergic component of neuroleptic receptors" and it was proposed as early as 1978 that this component may play an important role in the antipsychotic properties of neuroleptics (Leysen *et al.*, 1978). Nevertheless, as average clinical doses correlated better with D2 affinity rather than 5-HT_2 affinity, D2 receptor blockade was proposed to be the principal mechanism of action of neuroleptic drugs (Creese *et al.*, 1976; Peroutka and Snyder, 1980; Seeman and Lee, 1975). Drugs such as clozapine, chlorpromazine, thioridazine, and pipamperone had significantly higher affinity for 5-HT_2 than for D2 receptors. However, they were usually prescribed at high doses which induced D2 blockade, suggesting that 5-HT_2 blockade was not the principal mechanism of their antipsychotic action. The inverse was true for

haloperidol and fluphenazine, which did not significantly block 5-HT_2 receptors at average clinical doses (Peroutka and Snyder, 1980).

More recently, the demonstration of the superior efficacy of clozapine for treatment of schizophrenia and of its low incidence of EPS has promoted a renewed interest in the role of 5-HT_2 antagonism in schizophrenia. Given the lack of pharmacological specificity of clozapine, many theories have been proposed to account for its particular clinical profile and have been extensively reviewed elsewhere (Canton *et al.*, 1990; Deutch *et al.*, 1991; Lieberman, 1993; Meltzer, 1991; Seeman, 1992). Most prominent hypotheses include a higher *in vivo* selectivity of clozapine as compared to typical neuroleptics for (1) "corticolimbic" D2 receptors, as compared to "striatal" D2 receptors (Altar *et al.*, 1986; Deutch *et al.*, 1992), possibly as an effect of lower competition with endogenous DA in corticolimbic regions than in striatal regions (Seeman, 1990); (2) D4 receptors (Seeman, 1992; Van Tol *et al.*, 1991); (3) 5-HT_2 receptors (Altar *et al.*, 1986; Meltzer, 1989; Meltzer *et al.*, 1989; Rasmussen and Aghajanian, 1988). While a host of preclinical and clinical data support each of these assumptions, the introduction of new compounds with a more narrow pharmacological profile is indispensable to identify which of these putative mechanisms are critical for a clozapine-like atypical profile. For example, supporting the first hypothesis is the relative corticolimbic selectivity of benzamides with atypical profiles such as sulpiride or remoxipride, compounds which are otherwise devoid of D4 and 5-HT_2 selectivity as compared to D2. Compounds with high D4/D2 selectivity other than clozapine or pure D4 receptor antagonists have not been shown to be effective antipsychotics (Bristow *et al.*, 1997; Kramer *et al.*, 1997; Sanyal and VanTol, 1997). Many new compounds support the hypothesis that a relative 5-HT_2 to D2 selectivity provide "atypical" properties, the first one extensively tested being risperidone.

Following an extensive study of the *in vitro* receptor affinity profile of typical and putative atypical compounds, Meltzer *et al.* (1989) proposed that a ratio of 5-HT_2 pK_i to D2 pK_i > 1.19 (corresponding to a 25-fold selectivity for 5-HT_2 as compared to D2) was desirable to achieve an atypical profile. Risperidone, a compound not included in the original study of Meltzer *et al.* (1989) provides a 19-fold selectivity, slightly lower than the cutoff point originally proposed, but still more selective than the typical chlorpromazine (sevenfold selectivity). Placebo-controlled studies have demonstrated the antipsychotic efficacy of risperidone (Meco *et al.*, 1989; Mesotten *et al.*, 1989), and comparison studies with haloperidol or perphenazine have shown superior antipsychotic properties of risperidone (Claus *et al.*, 1992; Hoyberg *et al.*, 1993). The US–Canadian collaboration study included 388 schizophrenic patients divided in six groups: placebo, 2, 6, 10, or 16 mg daily risperidone and haloperidol 20 mg daily for 8 weeks (Marder and Meibach, 1994). Positive symptoms were significantly reduced as compared to placebo in the 6-, 10-, 16-mg risperidone groups and in the 20-mg

haloperidol group. Negative symptoms were significantly reduced only in the 6- and 16-mg risperidone group, but not in the 20-mg haloperidol group. EPS were significantly higher than placebo in the 16-mg risperidone group and in the 20-mg haloperidol group.

Because haloperidol is significantly more selective toward D2 than 5-HT_2 and because neither haloperidol nor risperidone have prominent antimuscarinic properties, this study provided the best data to date to evaluate the impact of the addition of a preferential 5-HT_2 blockade to D2 blockade in the treatment of schizophrenia. This study supports the hypothesis that a "balanced" 5-HT_2/D2 blockade has superior efficacy in the treatment of negative symptoms and a lower EPS liability. However, EPS and negative symptoms can be correlated and difficult to distinguish clinically. The observation of a significant improvement in negative symptoms despite a similar incidence of EPS in the 16-mg risperidone group as compared to the 20-mg haloperidol group suggests that improvement in negative symptoms is unrelated to decreased EPS. This study also indicates that 5-HT_2 blockade does not affect the incidence of EPS in the presence of complete or near complete D2 blockade. Other atypical agents have been introduced: olanzapine (Tollefson *et al.*, 1997), quetiapine (Arvanitis and Miller, 1997), and ziprasidone (Daniel *et al.*, 1999; Keck *et al.*, 1998; Seeger *et al.*, 1995). These have been demonstrated to be effective in the treatment of positive and negative symptoms in schizophrenia, with fewer side effects than typical neuroleptics. Most studies have shown a preferential response of negative symptoms to atypical antipsychotics versus typicals (Kane *et al.*, 1988; Tollefson *et al.*, 1997). This, however, was attributed by some investigators to an improvement in secondary negative symptoms, that is those related to positive symptoms, depression, EPS, or environmental deprivation, versus the primary negative symptoms otherwise characterized by the deficit syndrome, resulting in controversial debates (Meltzer, 1995). Meta-analyses of available studies have been published showing generally slight advantages of atypical antipsychotics in the treatment of negative symptoms (Leucht *et al.*, 1999). Despite the traditional resistance to treatment of cognitive impairments in schizophrenia, data related to atypical neuroleptics suggest that these drugs may have relatively greater efficacy than typical neuroleptics for treating these deficits (Meltzer and McGurk, 1999; Weinberger and Gallhofer, 1997). The improvement in negative symptoms and cognition is generally attributed to increased dopaminergic tone in the frontal cortex induced by 5-HT_{2A} antagonism, and possibly 5-HT_{1A} agonism for some compounds (see below).

While these treatment studies clarify the therapeutic effect of a combined 5-HT_2 and D2 antagonism, they do not inform us about the potential benefit of "pure" 5-HT_2 antagonists in the treatment of schizophrenia. Ritanserin, a potent 5-$HT_{2A/2C}$ antagonist, is not devoid of activity at the D2 receptor, but its 5-HT_2/D2 selectivity is three times higher than risperidone. In predominantly type II

schizophrenic patients, ritanserin augmentation of classical antipsychotics, as compared to placebo augmentation, was found to induce a significant reduction in BPRS mainly due to a decrease in negative symptoms such as anergia, anxiety/depression (Gelders, 1989). In this trial, ritanserin was more potent than placebo in reducing EPS, a finding which has been replicated (Bersani *et al.*, 1990; Miller *et al.*, 1990). However, therapeutic effects of ritanserin administered alone remain controversial and need further study (Bleekers *et al.*, 1990; Wiesel *et al.*, 1994). Pipamperone, a highly selective 5-HT_2/D2 drug, has been characterized by low EPS profile and antiautistic, disinhibiting, and resocializing effects (Gelders, 1989; Leysen *et al.*, 1978). MDL 100907, a compound with high affinity for 5-HT_{2A} receptors and negligible affinity for D2 receptors, has shown promising properties in preclinical studies predictive of atypical antipsychotic properties (Sorensen *et al.*, 1993). However, a therapeutic effect of MDL 100907 in phase II trials in patients with schizophrenia has not been demonstrated yet, despite many years of development so far, suggesting that pure 5-HT_{2A} antagonism alone may not be sufficient to have a clinically effective antipsychotic agent. D2 blockade remains so far a necessary component for a therapeutic antipsychotic effect, as demonstrated by the fact that no known effective antipsychotic lacks D2 antagonism. Clinical trials with fananserin, an antagonist at D4 and 5-HT_{2A} receptors, show lack of efficacy, illustrating the notion that neither D4 or 5-HT_{2A} antagonism, in the absence of D2 antagonism, seem to be associated with clinical improvement (Truffinet *et al.*, 1999). In conclusion, antagonism at 5-HT_{2A} when added to D2 antagonism may contribute to the atypical profile of an antipsychotic, that is, to better tolerability, fewer motor side effects, and better efficacy on negative symptoms, but alone, may not confer antipsychotic properties.

E. Action of Antipsychotic Drugs at Other Serotonergic Receptors

Most atypical antipsychotics have affinities for multiple serotonergic receptors, as summarized in Table II, the 5-HT_{1A}, 5-HT_{2C}, 5-HT_6, and 5-HT_7 deserve further discussion, as 5-HT_3 antagonists have been shown to lack antipsychotic property (Newcomer *et al.*, 1992). The actions of 17 antipsychotic agents at the 5-HT_{1A} were explored by (Newman-Tancredi *et al.*, 1998). Clozapine, ziprasidone, and quetiapine exhibited partial agonist activity and marked affinity at the human 5-HT_{1A} receptors, similar to their affinity at D2 receptors. In contrast, risperidone and sertindole displayed low affinity at 5-HT_{1A} receptors and behaved as "neutral" antagonists. Likewise the "typical" neuroleptics, haloperidol, pimozide, raclopride, and chlorpromazine exhibited relatively low affinity and "neutral" antagonist activity. This study suggests that agonist activity at 5-HT_{1A} receptors may be beneficial, although it is clear from clinical experience with drugs such as buspirone that 5-HT_{1A} agonism without D2 blockade does not

TABLE II
AFFINITIES OF SELECTED ANTIPSYCHOTIC DRUGS FOR 5-HT RECEPTORS

Drug	5-HT_{1A}	5-HT_{1B}	5-HT_{1D}	5-HT_{2A}	5-HT_{2C}	5-HT_3	5-HT_6	5-HT_7
Clozapine	132[a,*]	1200[b]	980[b]	4[c]	5[c]	69[b]	9.5[d]	6.3[e]
Olanzapine	1637[a,*]	1355[b]	800[b]	1.9[c]	2.8[c]	57[b]	10[d]	104[e]
Risperidone	292[a]	1325[b]	100[b]	0.39[c]	6.4[c]		2400[d]	1.39[e]
Quetiapine	250[a,*]	5400[b]	6220[b]	82[c]	1500[c]	170[b]	33[d]	
Sertindole	433[a]			0.2[c]	0.51[c]			
Ziprasidone	1.24[a,*]			0.25[c]	0.55[c]			
Amperozide	3100[c]			20[c]	440[c]		1600[d]	549[e]
Remoxipride	11000[c]			23000[c]	5500[c]			5000[e]
Haloperidol	1910[a]		6950[b]	28[c]	1500[c]	>1000[b]	6600[d]	263[e]
Aripiprazola	5.6[f]	830[f]	68[f]	22[f]	76[f]	630[f]	570[f]	10.3[f]

[a]Values taken from Newman-Tancredi *et al.* (1998), measured in cloned human receptors.
[b]Bymaster *et al.* (1996), in rat brain (Bymaster *et al.*, 1999).
[c]Values reported in Arnt *et al.* (1997), available from Lunbeck Pharmacological screening system (Arnt, 1998).
[d]Kohen *et al.* (1996), values obtained in human cloned receptors.
[e]Roth *et al.* (1994), in cloned rat receptors.
[f]Shapiro *et al.* (2003).
*Indicates an agonist effect at this receptor.

confer antipsychotic properties. Data has indicated that DA release in the frontal cortex induced by clozapine and other atypical antipsychotics is mediated by 5-HT_{1A} agonism (Rollema *et al.*, 1997). This provides a mechanism for a potential role of this receptor in alleviating negative symptoms and cognitive impairment, an important property of atypical antipsychotics. Currently, new compounds with strong affinity for this receptor are under development and may shed further light on its contribution to the treatment of schizophrenia.

Clozapine and olanzapine have high affinities for the newly discovered 5-HT_6 receptor ($K_{is} < 20$ nM), while clozapine and risperidone but not olanzapine displayed affinities for the 5-HT_7 receptor lower than 15 nM in cloned rat cells (Roth *et al.*, 1994). In addition, this study showed that several typical antipsychotic agents (chlorpromazine and fluphenazine) had high affinities for both the 5-HT_6 and 5-HT_7 receptors with pimozide displaying the highest affinity of all the typical antipsychotic agents tested for the 5-HT_7 receptor ($K_i = 0.5$ nM). This study seems to indicate that a high affinity for 5-HT_6 or 5-HT_7 does not relate to the atypical properties of antipsychotics, as it is shared by some of the typical antipsychotics, and is present in a minority of atypical antipsychotics, unlike 5-HT_{2A} antagonism.

Studies have shown that serotonin has opposing effects via different serotonergic receptor subtypes. A study by Martin *et al.* (1997) showed that ritanserin, a mixed 5-$HT_{2A/2C}$ antagonist, counteracts the inhibitory effect of MDL

100907 on the hyperlocomotion induced by MK-801 in a mouse model of schizophrenia. In another elegant series of experiments (Martin *et al.*, 1998), the same group showed that the effect of MDL 100907 was abolished by serotonin depletion achieved by pCPA treatment, and restored by restitution of endogenous serotonin. This finding suggests that activation of 5-HT_{2A} receptors is stimulatory while activation of 5-HT_{2C} receptors is inhibitory. A first conclusion from this study is that agonism at 5-HT_{2C} receptor may be antipsychotic. Another conclusion is that 5-HT_{2C} antagonist effects may depend on increased serotonergic tone. This would suggest that a response to atypical antipsychotics may be observed in patients with high serotonergic transmission. This conclusion is in agreement with clinical studies showing that a high serotonin tone, reflected by a low pretreatment CSF HVA/5HIAA ratio, predicts preferential response to clozapine (see references in Martin *et al.*, 1998). According to this line of thinking, agonism at the presynaptic 5-HT_{1A} receptor would result in a decreased serotonergic tone and would diminish the beneficial effects of 5-HT_{2A} antagonists, while therapeutic strategies aiming at increasing serotonergic tone, would be expected to enhance the efficacy of 5-HT_{2A} antagonists. An alternative interpretation of this data is that atypical antipsychotics may benefit most those patients without serotonergic deficits. Severe deficits in serotonergic function may underlie treatment resistance.

IV. 5-HT–DA Interactions Relevant to Schizophrenia

A. VTA DA Neurons Activity

Activity of DA neurons is inhibited by raphe stimulation. This effect is mediated by serotonin (Dray *et al.*, 1978; Fibiger and Miller, 1977) and by local dendritic release of DA (Nedergaard *et al.*, 1988; Williams and Davies, 1983) promoting D2 autoreceptor activation. In addition, this inhibitory effect of serotonin on Ventral Tegmental Area (VTA) and SN neurons may be mediated by 5-HT_2 receptors: acute systemic administration of the 5-HT_2 antagonist ritanserin increased the burst firing and firing rate of VTA and SN neurons (Ugedo *et al.*, 1989). This effect required the presence of intact endogenous 5-HT, as it was not observed after pCPA treatment. Thus, VTA and SN are under tonic inhibition by 5-HT neurons, possibly via DA dendritic release mediated by 5-HT_2 receptors. Acute administration of ritanserin increased DA concentration in the extracellular fluid in the accumbens measured with microdialysis (Devaud and Hollingsworth, 1991). Since extracellular DA concentration is thought to depend more on the tonic than the phasic release of DA (Grace, 1991), this observation suggests that 5-HT_2 antagonism increases the tonic release of DA in the terminal fields. Furthermore, VTA neurons are more sensitive than SN neurons to the disinhibiting effect of 5-HT_2 antagonists.

Low doses of ritanserin or ICS 169,369, a highly selective and potent 5-HT_2 antagonist, increase A10 firing rate but do not affect A9 cells. At higher doses, this selective effect of 5-HT_2 antagonists on VTA neurons is lost (Goldstein *et al.*, 1989).

In addition to interactions at the level of the VTA, 5-HT_2 receptors antagonism promotes DA release in the terminal fields of the VTA projection. In the prefrontal cortex, local administration of the 5-HT_{2A} antagonists clozapine, ritanserin, ICS 205,930, amperozide, and MDL 100907 increase DA efflux measured with microdialysis (Hertel *et al.*, 1996). Together, these observations suggest that 5-HT_2 blockade might enhance DA activity at the level of the VTA and the DA terminals.

Activation of VTA DA systems by 5-HT_2 blockade also prevents dysregulation of VTA DA functions observed following reductions in prefrontal cortex glutamatergic input. Local and reversible cooling of the prefrontal cortex alters the pattern of activity of VTA cells, from their normal, burst firing activity to a regular "pacemaker" pattern (Svensson and Tung, 1989). This alteration of firing activity has been proposed to mediate some of the negative symptoms associated with hypofrontality in schizophrenia such as poor drive and reward. Administration of the NMDA antagonist phencyclidine, known to induce both positive and negative symptoms in humans (Allen and Young, 1978; Snyder, 1980), produces the same effects as hypofrontality on VTA neurons, that is a reduction in burst activity (Svensson, 1993). Ritanserin and amperozide, both potent 5-HT_2 blockers, protect VTA DA cells from deactivation induced by cooling of prefrontal cortex or phencyclidine administration (Grenhoff *et al.*, 1990; Svensson, 1993; Svensson *et al.*, 1989, 1995). These observations are also compatible with a stimulating effect of 5-HT_2 antagonists on DA neuronal activity.

In summary, 5-HT_2 antagonists might reduce negative symptoms in schizophrenia through activation of midbrain DA projections to the limbic system and cerebral cortex. Since VTA DA neurons projecting to the accumbens are involved in drive and reward (Bozarth, 1986), it has been proposed that activation of VTA neurons by 5-HT_2 antagonists might provide a basis for their thymosthenic action and the improvement in negative symptoms (Ugedo *et al.*, 1989).

Behavioral studies performed with raphe lesions or pCPA treatment have consistently demonstrated an *enhancement* of amphetamine effects after 5-HT depletion. However, more recently, electrophysiological and microdialysis studies have demonstrated that selective 5-HT_2 blockade *decreases* amphetamine effects on locomotion. MDL 100907, a selective 5-HT_{2A} antagonist, blocks amphetamine stimulated locomotion at a dose that does not affect spontaneous locomotion (Sorensen *et al.*, 1993). While having no effect on basal DA extracellular concentration, 5-HT_2 antagonists such as MDL 100907, amperozide, and ketanserin decrease MDMA-mediated DA release measured by microdialysis

(Nash, 1990; Schmidt *et al.*, 1994). Electrophysiological data support the fact that 5-HT_2 blockade decreases DA mediated amphetamine effects by interfering with regulation of DA synthesis.

D-Amphetamine produces a marked inhibition of DA activity as recorded by single-cell recording. This inhibition is due to neural feedback loops in the SN and to the stimulation of somatodendritic receptors following DA release in VTA (Bunney and Aghajanian, 1976; Wang, 1981). α-Methyl-paratyrosine (αMPT), an inhibitor of tyrosine hydroxylase, the rate-limiting step in DA synthesis, attenuates amphetamine-induced DA release (Butcher *et al.*, 1988) and blocks amphetamine-induced slowing of cell firing (Bunney and Aghajanian, 1976). Thus, DA synthesis plays a major role in the effect of amphetamine on DA neurons. Interestingly, the selective 5-HT_2 antagonists ritanserin and MDL 28,133A also significantly suppress the effect of amphetamine on VTA neurons. This effect was, however, restored when L-dopa was coadministered with a 5-HT_2 antagonist. Since L-dopa enters the DA synthetic pathway beyond the point of synthesis regulation (tyrosine hydroxylase), it was proposed that 5-HT regulates tyrosine hydroxylase activity via 5-HT_2 receptors. While the 5-HT_2 agonist DOI hydrochloride does not appear to increase DA synthesis when given alone, it greatly potentiates amphetamine-induced increase in DA synthesis (Huang and Nichols, 1993) and amphetamine-induced DA release (Ichikawa and Meltzer, 1995). These data suggest that 5-HT_2 stimulation may be needed to maintain the increase in phasic DA neuronal activity, as observed after administration of stimulants or during stress. Thus, in this model, 5-HT_2 blockade would decrease DA phasic activity (a tyrosine hydroxylase dependent process) without affecting tonic basal DA activity (Schmidt *et al.*, 1993). Since positive symptoms are associated with increased DA release in schizophrenia (Laruelle *et al.*, 1996), these preclinical observations suggest that 5-HT_2 antagonism might have a beneficial effect on positive symptoms.

V. Discussions

The hypothesis that schizophrenia may be associated with decreased tonic DA activity and increased phasic activity (Grace, 1993) provides a framework in which the effects of a balanced 5-HT_2/D2 antagonism could be conceptualized. The tonic mode, or baseline mode, plays a role in motivation and drive, and appears to be regulated by a corticostriatal and cortico-VTA glutamatergic input. In contrast, the phasic mode is responsible for a rapid increase of DA in the synapse, in response to emotions or stress. One function of the tonic baseline activity is to regulate the sensitivity of the system to the phasic release of DA. A decrease in the tonic activity would result in an increased sensitivity of the

phasic activity. In schizophrenia, cortical lesions may induce a hypoactivity of the corticostriatal and corticolimbic glutamatergic projections, leading to a decrease in the tonic release of DA, which may be associated with negative symptoms such as lack of drive and motivation. This decreased tonic activity in turn induces a state of hypersensitivity of the DA system to the phasic release, which can be the substrate of positive symptoms (Grace, 1993).

Examination of 5-HT-DA interactions mediated by the 5-HT_{2A} receptors lead to the following suggestion regarding the mechanism of action of "balanced" 5-HT_{2A}/D2 agents. It is suggested that 5-HT_{2A} blockade acts as a buffer to narrow the range of DA activity in the VTA projection territories, elevating the baseline activity (thus reducing negative symptoms) and decreasing the amplitude of the phasic reactivity (thus reducing positive symptoms). Decreased tonic activity of VTA is observed after inactivation of the frontal cortex or administration of NMDA antagonists. 5-HT_{2A} antagonists restore normal VTA DA activity after inactivation of the frontal cortex, and this might account for improvement in negative symptoms. Obviously, if stimulation of mesocorticolimbic DA baseline activity is the mechanism mediating the improvement in negative symptoms attributed to 5-HT_{2A} antagonism, this effect would be lost in the presence of a complete blockade of D2 receptors (Kapur and Remington, 1996). Thus, a moderate rather than a complete D2 receptors occupancy might be desirable to permit 5-HT_{2A} antagonism to exert its therapeutic action on the negative symptoms.

On the other hand, positive symptoms could be reduced by attenuation of DA phasic activity via blockade of 5-HT_{2A}-stimulated tyrosine hydroxylase activity. If increased DA phasic activity is at least partially dependent on 5-HT_{2A}-stimulated tyrosine hydroxylase activity, 5-HT_2 blockade might reduce the tyrosine hydroxylase dependent DA phasic release responsible for the positive symptoms of schizophrenia. One study seems to contradict this hypothesis so far, where clozapine and risperidone treatment did not normalize the amphetamine-induced DA release in patients with schizophrenia compared to controls (Breier *et al.*, 1999). However, the effect of D2 blockade may have counfounded the interpretation of the effect of the 5-HT_2 antagonism in this study. Revisiting this issue after treatment with a pure 5-HT_2 antagonist such as MDL 100907 is warranted.

So far, 5-HT_{2A} antagonists show modest efficacy as stand-alone treatments for schizophrenia. However, they do not appear to be as effective in treating schizophrenia as haloperidol. In addition, it is now clear that 5-HT_{2A} antagonists do not increase the D2 receptor blocking threshold associated with emergence of EPS since the threshold of D2 receptor occupancy associated with EPS is not markedly different between these drugs and drugs devoid of 5-HT_{2A} antagonism (Kapur *et al.*, 1995, 1998; Knable *et al.*, 1997; Nyberg *et al.*, 1993). The benefit derived from the combination of 5-HT_{2A} antagonism with a partial D2 blockade may relate to the following two factors: (1) 5-HT_{2A} antagonism increases DA release in the

cortex, this effect might lead to an improvement in negative symptoms and cognition, possibly through an increased stimulation of the D1 receptor. This effect may be potentiated by agonism at the 5-HT_{1A} receptor. (2) 5-HT_{2A} antagonists may decrease the tyrosine hydroxylase dependent, or phasic, DA release in subcortical areas and improve positive symptoms, 5-HT_{2A} antagonists may be even more beneficial in presence of high serotonergic tone, since increased stimulation of the 5-HT_{2A} receptors, because of their location on apical dendrites of most pyramidal cells in the cortex, may lead to a dysregulation of glutamatergic transmission and corticocortical as well as corticosubcortical transmission. As we understand better the role of the 5-HT_{2A} receptors in the treatment of schizophrenia, much remains to be learned about the other receptors, although the evidence so far does not suggest they play a role as prominent as the 5-HT_{2A}. More research is needed to further clarify the role of these receptors.

In conclusion, results of postmortem studies indicate possible alteration of 5-HT transmission in the prefrontal cortex of patients with schizophrenia. Decreased 5-HT transporter density, increased 5-HT_{1A} receptors, and decreased 5-HT_{2A} receptors have all been suggested by postmortem studies in schizophrenia, but these findings have not been consistently replicated and have not yielded conclusive evidence so far. On the other hand, stimulation of 5-HT_{2A} receptors is psychotogenic, although LSD-induced psychosis is an imperfect model of the illness. 5-HT_{2A} receptor blockade is a useful augmentation or modulation of D2 receptor blockade, but 5-HT_{2A} antagonism alone has not yet demonstrated incisive antipsychotic properties. If 5-HT_{2A} blockade per se does not produce antipsychotic effects in patients with schizophrenia, it would support the argument that these symptoms are not primarily due to hyperstimulation of 5-HT_{2A} receptors. The potential of pharmacological interventions targeted at other 5-HT receptors (such as 5-HT_{1A} agonism) remains to be clarified. Overall, a comprehensive model of alterations of 5-HT transmission in schizophrenia has not yet emerged and additional research is needed, not only to clarify possible alterations of 5-HT systems in schizophrenia but also to establish their significance in terms of modulation of other transmitters systems (DA and glutamate), role in symptomatology, and treatment opportunity.

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SEROTONIN AND DOPAMINE INTERACTIONS IN RODENTS AND PRIMATES: IMPLICATIONS FOR PSYCHOSIS AND ANTIPSYCHOTIC DRUG DEVELOPMENT

Gerard J. Marek

Eli Lilly and Company, Lilly Research Laboratories, Psychiatric Disorders Discovery Biology
Lilly Corporate Center, Indianapolis, Indiana 46285, USA

Since the late 1950s, appreciation of dopamine receptor blockade has played a primary role in understanding the mechanism underlying the therapeutic effects of antipsychotic drugs in schizophrenic patients in treating the positive symptoms of schizophrenia (e.g., delusions and hallucinations). Development of the second generation of antipsychotic drugs, otherwise known as atypical antipsychotic drugs, has resulted in treatments with improved subjective tolerability but relatively modest improvements in the negative symptoms of schizophrenia such as avolition, flat affect, and anhedonia. The major current challenge is to develop medications which can further improve negative symptoms treatment and also tackle the intractable clinical problems of cognitive impairment associated with schizophrenia. Further advances along these lines with respect to the dopaminergic and serotonergic neurostransmitter systems will be aided by an appreciation of the interaction between dopamine and serotonin receptor subtypes in a range of key brain structures, such as the prefrontal cortex, thalamus, striatum, amygdala, hippocampus, and the brain stem nuclei, from which the cell bodies of monoaminergic-containing neurons originate. Increasing emphasis on the use of animal models which are homologous to critical aspects of the pathophysiology

INTERNATIONAL REVIEW OF NEUROBIOLOGY, VOL. 78
DOI: 10.1016/S0074-7742(06)78006-0

0074-7742/07 $35.00

in the brains of schizophrenic patients will also be required, especially as negative symptoms and cognitive impairment become an important focus for generating novel therapeutics.

I. Introduction

Serotonin and dopamine represent two of the three monoaminergic neurotransmitter systems that play prominent roles in the action of most psychotropic drugs used for the treatment of major neuropsychiatric syndromes. Dopamine, especially, has played a critical role in therapeutics for schizophrenia since the initial hypothesis by Carlsson that blockade of dopamine receptors plays a role in their therapeutic effects for psychotic patients (Carlsson and Lindquist, 1963). This initial hypothesis has evolved over the last several decades to emphasize a relative hypofunctional state in the prefrontal cortex and a hyperfunctional state in the striatum (Abi-Dargham, 2004; Laruelle *et al.*, 2005; Winterer and Weinberger, 2004). The emergence of human PET studies of receptor occupancy demonstrated that occupancy of ~65% of dopamine D2 receptors is associated with the therapeutic effects of antipsychotic drugs (Farde *et al.*, 1992; Kapur *et al.*, 1999) except for clozapine and aripiprazole. Antipsychotic drug development in the last 15 years has emphasized serotonin (5-hydroxytryptamine, 5-HT) and 5-HT_{2A} receptor blockade as a mechanism to either minimize extrapyramidal symptoms (EPS) or increase efficacy for psychotic symptoms, negative or depressive symptoms, and cognitive dysfunction (Meltzer, 1999; Meltzer *et al.*, 1989). This has resulted in a number of "atypical" antipsychotic drugs or second generation antipsychotics (SGA). Here we discuss the underlying neurobiology for the dopamine and serotonergic neurotransmitter systems, their interactions, and gaps in our knowledge toward developing better therapeutic agents for schizophrenia. A large literature has developed describing interactions of dopamine and 5-HT regarding psychomotor stimulant drug effects, so understanding these relationships will be emphasized. A number of cortical and subcortical structures have been implicated in schizophrenia. Since structural changes and/or dysfunction of the prefrontal cortex (Selemon and Goldman-Rakic, 1999; Selemon *et al.*, 1995), thalamus (Clinton and Meador-Woodruff, 2004), dorsal and ventral striatum (nucleus accumbens, n. accumbens), and the hippocampal formation (Harrison, 2004) play prominent roles from postmortem studies of schizophrenic patients and neuroimaging studies from first-break schizophrenic patients, we will focus attention on how dopamine and 5-HT interact to modulate the function of these macrocircuits, with a special emphasis on the prefrontal cortex. Other regions are implicated in the neurodevelopmental changes, and

pathophysiology of schizophrenia such as the amygdala or the cerebellum will not be discussed in this chapter.

II. Dopamine and 5-HT Receptors

Dopamine has a neuromodulatory influence on neurotransmission in the brain by acting on five different dopamine G-protein–coupled receptor subtypes (Neve *et al.*, 2004) that are defined by both a dopamine D1-like (dopamine D1 and D5 receptors) and dopamine D2-like (dopamine D2, D3, and D4 receptors). The D1-like receptors typically couple to $G_{\alpha s}$ and $G_{\alpha olf}$ which leads to sequential activation of adenylyl cylcase, cyclic AMP-dependent protein kinase, and the protein phosphatase-1 inhibitor DARPP-32. This leads to pleiotropic effects on receptors, enzymes, ion channels, and transcription factors. Dopamine D1 receptors may also couple to phospholipase C. The activation of the phosphoinositide pathway and cAMP-dependent mobilization of intracellular Ca^{2+} is responsible for other signaling properties. For example, activation of either dopamine D1 or D5 receptors, if coexpressed with calcyon, can stimulate calcium release from intracellular stores after the cell has been primed by activation of $G_{\alpha q}$-coupled receptors (Lezcano *et al.*, 2000). This type of interaction appears to be region specific as it occurs in the neocortex and hippocampus but not the striatum (Lezcano and Bergson, 2002). The dopamine D2-like receptors (dopamine D2, D3, and D4) couple to $G_{\alpha i}$ and $G_{\alpha o}$ heterotrimeric G-proteins to decrease activity of adenylyl cyclase but also regulate other effectors such as ion channels, phospholipases, protein kinases, and receptor tyrosine kinases via $G_{\beta\gamma}$ subunit interactions. These differential effects on postreceptor transduction pathways for the D1-like and D2-like receptor families emphasize that the effect of either increasing or decreasing dopaminergic transmission in a given region of the brain is dependent on the receptors activated by tonic (volume transmission) or phasic dopamine input (synaptic transmission) and the type of neuron (glutamatergic projection cell or GABAergic interneuron or projection cell) being affected (Onn *et al.*, 2006). Further complexity in dopamine receptor signaling due to protein–protein interactions (e.g., receptor oligomerization or receptor interactions with scaffolding and signal-switching proteins) are being uncovered (Bergson *et al.*, 2003; Neve *et al.*, 2004).

Serotonin has a neuromodulatory influence on neurotransmission in the brain and targets in the periphery by acting on at least 15 different 5-HT receptor subtypes (Aghajanian and Sanders-Bush, 2002; Barnes and Sharp, 1999) that have been classified according to 7 different families according to coupling to ion channels (5-HT_3 receptors) or heterotrimeric G-proteins (5-$HT_{1A/1B/1D/1E/1F}$, 5-$HT_{2A/2B/2C}$, 5-HT_4, 5-$HT_{5A/5B}$, 5-HT_6, and 5-HT_7 receptors). The 5-HT_1

family of receptors is negatively coupled to adenylyl cyclase through $G_{\alpha i}$- and $G_{\alpha o}$-containing G-proteins. The 5-HT_5 family may also share this coupling. In contrast, the 5-HT_4, 5-HT_6, and 5-HT_7 receptors are positively coupled to adenylyl cyclase through $G_{\alpha s}$. The 5-HT_2 family of receptors are coupled to phosphoinositide turnover and the arachidonate pathway via $G_{\alpha q}/G_{\alpha 11}$-containing G-proteins that are linked to phospholipases C and A2, respectively. The 5-HT_3 receptor is directly coupled to ion channels and is the only ionotropic monoaminergic receptor.

When considering dopamine and serotonin interactions at a cellular level, activation of dopamine D1-like or 5-$HT_{2A/2B/2C}$, 5-HT_4, 5-HT_6, and 5-HT_7 receptors could potentiate the action of the other transmitter where these receptors are colocalized in the same cells with similar transduction pathways. Such a convergence of transduction pathways leading to an integrated modulation of DARPP-32 has been demonstrated in the rodent forebrain (prefrontal cortex, n. accumbens, neostriatum) for activation of dopamine D1, 5-HT_2, 5-HT_4, and 5-HT_6 receptors (Nishi *et al.*, 2000; Svenningsson *et al.*, 2002). Similarly, the dopamine D2-like and the 5-HT_1 receptor family and 5-HT_5 receptors could also potentiate the action of either dopamine or serotonin when these receptors are colocalized in the same cells with similar transduction pathways. Conversely, opposing effects of these neurotransmitters might be present where these receptors are localized to a glutamatergic projection cell or GABAergic interneuron, respectively. The later sections discussing the monoamine nuclei, the thalamus, the prefrontal cortex, the striatum, and the hippocampus will discuss the known relationships of these neurotransmitters and will also point out critical gaps to understanding the monoaminergic transmitter interactions.

III. Psychomotor Stimulants: A Dopamine–Serotonin Interaction "Case Study"

Prior to the elucidation of multiple dopamine and 5-HT receptors, a substantial body of work had arisen to suggest that decreasing serotonergic neurotransmission increased the behavioral effects of amphetamine, the direct acting dopamine agonist apomorphine, and dopamine (Breese *et al.*, 1974; Campbell and Fibiger, 1971; Carter and Pycock, 1978, 1979; Fuxe *et al.*, 1975; Green and Harvey, 1974; Hollister *et al.*, 1974; Lucki and Harvey, 1979; Neill *et al.*, 1972; Segal, 1976). Conversely, increasing serotonergic neurotransmission decreased the behavioral effects of amphetamine, apomorphine, and dopamine (Breese *et al.*, 1974; Carter and Pycock, 1978; Warbritton *et al.*, 1978).

Paradoxically, *in vivo* microdialysis studies have found that acute administration of the selective serotonin reuptake inhibitor (SSRI) fluoxetine with the catecholamine-enhancing antidepressant bupropion appears to result in a

synergistic increase in extracellular dopamine in the prefrontal cortex and n. accumbens compared to either agent alone (Li *et al.*, 2002). Similar work suggesting at least an additive effect between dopamine and 5-HT were revealed by the locomotor stimulation observed for a combination of the SSRI fluovoxamine and the nonselective reuptake inhibitor mazindol despite both drugs alone having either had no effect (mazindol) or decreasing locomotor activity (fluvoxamine). The attenuation of fluvoxamine/mazindol-induced hyperactivity by the 5-HT_{2A} receptor antagonist M100907, and potentiation of fluoxamine/mazindol hyperactivity by the 5-$HT_{2B/2C}$ receptor antagonist is consistent with reciprocal interactions of 5-HT_{2A} and 5-HT_{2C} receptors (McMahon and Cunningham, 2001b) discussed below. Clearly, different 5-HT receptors may mediate differential effects on dopamine function. These conflicting sets of observations exploring interactions of 5-HT and dopamine at mediating the effects of psychomotor stimulants or monoamine reuptake inhibitors may need to take into account activation of different 5-HT receptor subtypes with respect to tonic 5-HT release (volume transmission) versus phasic 5-HT release (direct synaptic transmission).

While the pharmacological effects of cocaine are frequently attributed to effects on dopamine, the ability of cocaine to inhibit 5-HT reuptake (Andersen, 1989; Gatley *et al.*, 1996) and interactions between 5-HT and the dopaminergic system also appear to be relevant for understanding the psychopharmacology of cocaine. Reciprocal interactions between dopamine and 5-HT at a global level would appear to play a role in the ability of the monoamine reuptake inhibitor cocaine to decrease locomotor activity in dopamine (DAT) knockout mice (Gainetdinov *et al.*, 1999). In DAT knockout mice, a tonic elevation of extracellular dopamine is thought to mediate the increase in locomotor activity in comparison to wild-type mice. However, in naïve rats, blockade of 5-HT_{2A} receptors attenuates the psychomotor activation induced by either amphetamine or cocaine (McMahon and Cunningham, 2001a; O'Neill *et al.*, 1999). Similar to effects reported above with SSRIs and the uptake inhibitor mazindol, there also appear to be opposing effects of 5-HT_{2A} versus 5-HT_{2C} receptor activation or blockade on cocaine hyperactivity or self-administration (Filip *et al.*, 2004; Fletcher *et al.*, 2002b; McMahon *et al.*, 2001).

Positive modulation of cocaine-induced hyperactivity or self-administration also appears with activation of 5-$HT_{1A/7}$ receptors by 8-OH-DPAT (De La Garza and Cunningham, 2000). Similarly, activation of 5-HT_{1B} receptors in the ventral tegmental area (VTA) also increases cocaine-induced extracellular dopamine in the ipsilateral n. accumbens (O'Dell and Parsons, 2004). Overexpression of 5-HT_{1B} receptors in the VTA–n. accumbens pathway increases cocaine-induced locomotion and results in a leftward shift for the cocaine-induced conditioned place preference (Neumaier *et al.*, 2002). These effects are consistent with the absence of cocaine-induced place preference in 5-HT_{1B} receptor knockout mice (Belzung *et al.*, 2000). In contrast to cocaine-induced

effects, 5-HT_{1B} receptor activation in the n. accumbens decreases amphetamine self-administration. This discrepancy between the effects of 5-HT_{1B} receptor activation may be dependent on the presence of 5-HT_{1B} receptors in different cellular compartments (Fletcher *et al.*, 2002a). Pharmacological *blockade* of 5-HT_4 receptors in the n. accumbens shell attenuates cocaine-induced hyperactivity (McMahon and Cunningham, 1999). While 5-HT_6 receptor antagonism does not alter cocaine effects, amphetamine-induced behavioral effects were potentiated (Frantz *et al.*, 2002). Thus, activation of a number of serotonin receptors (5-$HT_{1A/7}$, 5-HT_{1B}, 5-HT_{2A}, 5-HT_4) does appear to mediate, in part, the behavioral and neurochemical effects of psychomotor stimulants. However, there also appears to be particular 5-HT receptors (e.g., 5-HT_{2C} receptors) which oppose the stimulating effects of amphetamines or cocaine.

IV. Monoaminergic Nuclei Interactions

Dopamine-containing cell bodies are localized primarily in the substantia nigra pars compacta (SNpc) and the VTA of the midbrain (Moore and Bloom, 1978). The SNpc projects to the dorsal striatum (caudate and putamen) and makes up the nigrostriatal dopaminergic system. The VTA has relatively precise topographical relationships with the prefrontal cortex (mesocortical dopaminergic system), the ventral striatum (n. accumbens), and other areas (thalamus, hippocampus, lateral septum, amygdala). Together, the projections to the n. accumbens and the other limbic structures outside of the prefrontal cortex make up the mesolimbic dopaminergic system. These nuclei which contain dopaminergic cells are not homogenous with respect to cell type. At least 15–20% of the neurons within the VTA are known to be GABAergic interneurons, and at least a portion of these are projection GABAergic interneurons to the prefrontal cortex (Carr and Sesack, 2000) and the n. accumbens (Van Bockstaele and Pickel, 1995). While these general relationships hold, it has also been proposed that the dopaminergic afferents to the primate thalamus may represent an additional distinct system involving cell bodies of origin in the hypothalamus, periacqueductal gray (PAG) matter, ventral mesencephalon, and the lateral parabrachial nucleus (Sanchez-Gonzalez *et al.*, 2005).

Serotonin-containing cell bodies which project to the limbic forebrain are found primarily in the dorsal raphe nucleus (DR) and the median raphe nucleus (MR) in the pons (Hensler, 2006). The DR projects to the prefrontal cortex, the lateral septum, ventral hippocampus, and n. accumbens as well as providing a relatively strong innervation to the substantia nigra pars reticulate (a nuclei containing the dendrites of SNpc cells) and VTA. The MR projections include the dorsal hippocampus, medial septum, and hypothalamus. Some areas such as

the intralaminar and midline thalamic nuclei receive a projection from both the DR and the MR (Azmitia, 1978). The midbrain raphe nuclei, like the midbrain SNpc and VTA, contain GABAergic interneurons. There is some evidence that at least some of these GABAergic interneurons may also be projection GABAergic interneurons (O'Hearn and Molliver, 1984; Van Bockstaele *et al.*, 1993). An interesting feature about the raphe nuclei is that most forebrain projection areas send back reciprocal projections via the habenula nuclei. However, the prefrontal cortex appears to be an area with a privileged direct projection to the dorsal raphe (Aghajanian and Wang, 1977; Hajos *et al.*, 1998; Martin-Ruiz *et al.*, 2001a; Peyron *et al.*, 1998; Sesack *et al.*, 1989).

The dorsal raphe nucleus also sends a dense serotonergic projection to the pars reticulata of the substantia nigra (SNr; Fallon and Loughlin, 1995). Dorsal raphe stimulation mainly induces inhibitory effects in SNpc cells, though non-5-HT afferents may be involved (Gervais and Rouillard, 2000). Both the dorsal raphe and the median raphe send axons which arborize in the VTA (Azmitia, 1978; Vertes, 1991; Vertes *et al.*, 1999). In turn, the VTA and cells from the A11 hypothalamic cell group project back to the dorsal raphe (Kalen *et al.*, 1988; Peyron *et al.*, 1995). While activation of both dopamine D1-like and D2-like receptors increase the firing rate of serotonergic-containing DR neurons and increase 5-HT release in the DR and striatum, it appears that dopamine D2 receptors outside of the DR are involved in these effects (Martin-Ruiz *et al.*, 2001b). GABAergic neurons in the PAG-expressing dopamine D2 mRNA are among candidate regions mediating this effect.

Regarding dopamine–serotonin interactions, multiple 5-HT receptors have different, and apparent, opposing influences on the presynaptic side of the dopaminergic system. There is a well-documented effect of 5-HT_{2C} receptor stimulation activating GABAergic cells in the SN and VTA, and thereby inhibiting frontocortical and accumbal dopaminergic transmission. Conversely, blockade of 5-HT_{2C} receptors increases activity of dopamine-containing cells in the VTA, and thereby increases dopaminergic transmission in the mesolimbic and mesocortical dopamine pathways (De Deurwaerdere *et al.*, 2004; DiMattio *et al.*, 2001; Millan *et al.*, 2000). Furthermore, *in vivo* evidence suggests that 5-HT_{2C} receptors inhibit dopamine release in the rat striatum and n. accumbens via constitutive activity (De Deurwaerdere *et al.*, 2004).

While activation of the 5-HT_{2C} receptor appears to play an inhibitory role on dopaminergic neurotransmission, activation of another receptor from the same family, the 5-HT_{2A} receptor, appears to facilitate dopaminergic neurotransmission in the mesolimbic and mesocortical pathways. For example, 5-HT_{2A} receptor mRNA tends to colocalize with a fraction of the dopamine-containing cells in regions of the VTA that preferentially projects to terminal fields of the mesolimbic pathway (Nocjar *et al.*, 2002). An ultrastructural study has found that 5-HT_{2A} receptors are present in the dendrites and soma of VTA dopamine-containing

cells (Doherty and Pickel, 2000). There is also a significant amount of functional data (discussed above) suggesting that 5-HT_{2A} receptor activation plays a role in the psychomotor stimulant effects of cocaine and amphetamine by enhancing extracellular levels of dopamine. The increase seen in extracellular DA following local infusion into the prefrontal cortex with the highly selective 5-HT_{2A} receptor antagonist suggests a facilitatory role for this receptor in the mesocortical pathway (Pehek *et al.*, 2001). Activation of cortical 5-HT_{2A} receptors also appears to increase stress-induced dopamine release in the mPFC (Pehek *et al.*, 2006). This effect may be mediated by polysynaptic neuronal circuits involving cortical pyramidal cells with 5-HT_{2A} receptors in the apical dendritic field (Bortolozzi *et al.*, 2005). The immunohistochemical localization of 5-HT_{2A} receptors in presumed monoaminergic axons of the prefrontal cortex might be an additional or alternative substrate for these latter findings (Miner *et al.*, 2003). A previous electron microscopic study had observed the presence of 5-HT_{2A} receptor immunoreactivity in small unmyelinated axons of the VTA that were likely either dopaminergic axons or axons from nondopaminergic cells coursing through the VTA (Doherty and Pickel, 2000). These opposing effects of 5-HT_{2A} versus 5-HT_{2C} receptors on dopaminergic neurotransmission highlight the critical importance of understanding the localization of different 5-HT receptors, SERT, and DAT with respect to both microcircuits and macrocircuits involving corticostriato-thalamic pathways.

5-HT_{1A} receptor partial agonist action has been discussed for a number of years with respect to development of antidepressant drugs by understanding the relative role of somatodendritic 5-HT_{1A} autoreceptors and 5-HT_{1A} postsynaptic receptors. There are a number of compounds currently in development possessing partial agonist action at 5-HT_{1A} receptors in addition to effects at other monoaminergic neurotransmitters such as dopamine D2/D3 receptors.

V. Serotonin and Dopamine in the Thalamus

The serotonergic innervation of the thalamus from the dorsal and median raphe is mainly distributed to the midline and intralaminar thalamic nuclei, the so-called "nonspecific" thalamic nuclei of Lorente de No. While the afferent projection from the dorsomedial nucleus (MD n.) of the thalamus to layer III of the neocortex is much discussed as a defining feature of the prefrontal cortex, the much less studied midline and intralaminar thalamic nuclei have a number of neuroanatomical relationships that make them critical structures with respect to major neuropsychiatric syndromes and therapeutics (Van der Werf *et al.*, 2000, 2002). First of all, these midline and intralaminar thalamic nuclei project to layers I and Va throughout the prefrontal cortex providing discrete laminar inputs to

the distal dentritic tuft and proximal apical dendrites of the principal output cells, the layer V pyramidal cells. The importance of the thalamus in coordinating higher functions of the cortex may be suggested by the tenfold greater number of corticothalamic fibers than thalamocortical fibers; the layer V pyramidal cells project back to the intralaminar and midline thalamic nuclei. Second, the intralaminar and midline thalamic nuclei make up the only projection from the thalamus to the dorsal and ventral striatum. Third, the midline and intralaminar thalamic nuclei also project to the amygdala, subiculum and return a principal projection from the brain stem reticular activating system. While the intralaminar and midline thalamic nuclei express a rich distribution of 5-HT_{2C} and 5-HT_{1B} receptor mRNA in the adult rat, activation of 5-HT_{2A} receptors appear to play an important role in inducing glutamate release from these afferents in layer I and Va of the prefrontal cortex (Aghajanian and Marek, 1997; Marek *et al.*, 2001). Thus, these anatomical relationships define a critical feature for the intralaminar and midline thalamic nuclei consistent with their physiological importance in mediating arousal and vigilance (Kinomura *et al.*, 1996).

While the serotonergic and noradrenergic innervation of the thalamus is much more prominent than that of dopamine, there is also evidence for physiological effects of dopamine within the thalamus. There is evidence for dopamine D2/D3 receptor binding in the human midline and intralaminar thalamic nuclei which is most intense for the paraventricular thalamic (PVT) nucleus (Rieck *et al.*, 2004). In addition to dopaminergic afferents to the PVT, there also appears to be a role for dopamine D2 receptor stimulation in modulating the MD n. Dopamine D2 receptor activation appears to increase the excitability of rat MD n. cells recorded using an *in vitro* preparation (Lavin and Grace, 1998). This direct effect of dopamine in the MD n. could have even a greater effect on distinct thalamocortical pathways given the convergence of axon terminals from the MD n. and the VTA (Kuroda *et al.*, 1996). However, it should also be noted that most of the dopaminergic cells of origin which project to the rodent MD n. and the PVT appear to arise from the hypothalamus rather than the VTA. In the primate, there does appear to be a projection from the SNr to the MD n., although the neurotransmitter phenotype of these cells has not been identified. In the primate, DAT immunopositive labeling has clearly suggested that dopamine does innervate the MD n. (Melchitzky and Lewis, 2001). As mentioned earlier, the heterogeneity of dopamine innervation to different regions in the thalamus has prompted one group to suggest that the "thalamic dopaminergic system" may be a novel system with respect to the classical recognition of the nigrostriatal, mesocortical, and mesolimbic dopaminergic systems (Sanchez-Gonzalez *et al.*, 2005). With respect to the potential complementary roles played by 5-HT_{2A} receptor activation versus dopamine D2 receptor activation on thalamocortical pathways, it is interesting that dopamine receptor activation, unlike 5-HT_{2A} or α_1 adrenergic receptor activation, does not appear to play a role at inducing excitatory postsynaptic

currents (EPSCs) recorded from layer V pyramidal cells, which appear to arise in part from the midline and intralaminar thalamic nuclei (Marek and Aghajanian, 1999).

VI. Dopamine and Serotonin in the Striatum

The effects of dopamine and serotonin in the striatum cannot be addressed without a brief description of the basal ganglia microcircuitry and macrocircuitry (Wilson, 1998). The neostriatum consists of dorsal striatum (caudate/putamen) and ventral striatum (n. accumbens). Increased dopaminergic neurotransmission in circuits running through the dorsal striatum is associated with motor stereotypies such as those induced by high dose-amphetamine treatment. The caudate/putamen receives inputs from sensory cortex, motor cortex, and prefrontal cortex that converge with projections from the intralaminar thalamic nuclei, dopaminergic inputs from the SNpc, and serotonergic inputs from the (DR). The GABAergic, substance P-containing medium spiny cells of the caudate/putamen then project to two major regions: the globus pallidus [external (GPe) and internal (GPi) segments] and the pars reticulata of the SNr. The "direct pathway" leading from the GPi and the SNr projects to the ventral thalamic nuclei tier, lateral habenula nuclei, and the deep layers of the superior colliculus. The "indirect pathway" involves GABAergic enkephalin-containing neostriatal medium spiny cells of the caudate/putamen which project to the external segment of the globus pallidus, which in turn projects to the subthalamic nucleus. It should be noted that the neostriatum does not provide a direct reciprocal projection back to the neocortex and the thalamus.

Enhanced dopaminergic neurotransmission in circuits running through the ventral striatum (n. accumbens) is associated with stimulation of increased locomotor activity induced by relatively low-dose amphetamine treatment. The n. accumbens core, unlike the dorsal striatum, receives serotonergic input via the median raphe. There are important topographical relationships of different areas of the prefrontal cortex, amgydala, thalamic, and hippocampus projecting onto the accumbal patch and matrix areas (Groenewegen *et al.*, 1999). For example, the n. accumbal patches project to the SNpc while the matrix areas project to the SNr. These patch (striosome) and matrix regions also have substantial differences with respect to μ-opioid receptor localization, calcium-binding proteins, and acetylcholinesterase [patch neuropil high in μ-opioid receptors; low in calbindin; acetylcholinesterase rich (Herkenham and Pert, 1981)]. Enkephalin and substance P have a more complex distribution, though they are differentially expressed in the patch or matrix in different regions of the neostriatum. The patch versus matrix distinction has important relationships to macrocircuitry afferent

relationships. For example, the n. accumbens core matrix receives projections from layer III or superficial layer V pyramidal cells in the prefrontal cortex in addition to strong afferents from a number of midline and intralaminar thalamic nuclei. The n. accumbens core patches receive projections from deep layer V pyramidal cells in the PFC (Gerfen, 1989) and amygdala but are relatively sparse with respect to thalamic afferents. However, it is important to note that the topography of afferents to the striatum from different prefrontal cortical layers to the striosomes (patch) or matrix can switch in different regions of the caudate/putamen (Ragsdale and Graybiel, 1990). One difference between the patch (striosome) and matrix region in the human caudate and putamen is that 5-HT_{2A} receptor binding appears more intense in the patch regions (Lopez-Gimenez *et al.*, 1999; Waeber and Palacios, 1994). 5-HT_{1A} receptor binding in the primate striatum also is more intense in the striosome or patch neuropil (Frechilla *et al.*, 2001; Mengod *et al.*, 1996).

This mosaic organization of the neostriatum and accumbens was previously identified in primates. The patch or striosome is equivalent to the cell islands while the matrix is similarly named in the primate (Goldman-Rakic, 1982). In the rodent, patterns of fos immunopositive cells have been studied with respect to predicting extrapyramidal side effects consistent with typical versus atypical antipsychotic drugs. The ratio striosome/matrix cells expressing fos immunopositive cells is greater for atypical antipsychotic drugs than for typical antipsychotic drugs such as haloperidol (Bubser and Deutch, 2002). However, single serotonin receptor subtypes such as the 5-HT_{1A} or 5-HT_{2A} receptor did not appear, by themselves, to account for these effects. These striatal compartments are differentially modulated under other circumstances. For example, the matrix compartment of the rat is preferentially activated during free movement, gentle restraint, or focal tactile stimulation during gentle restraint (Brown *et al.*, 2002). Conversely, stereotypy induced by cocaine or amphetamine or psychostimulant-induced sensitization appears to be associated with preferential early gene expression in the striosome compartment of the striatum (Canales and Graybiel, 2000; Capper-Loup *et al.*, 2002).

Dopamine can differentially affect the direct and indirect pathways of the striatum. Dopamine D1 receptors are preferentially expressed in the substance P-containing striatonigral direct pathway whereas dopamine D2 receptors are preferentially expressed on the enkephalin-containing spiny neurons making up the striatopallidal indirect pathway (Gerfen *et al.*, 1990; Surmeier *et al.*, 1996). Thus, dopamine would tend to enhance neurotransmission through the direct pathway while attenuating transmission in the indirect pathway. However, there is also a subpopulation of striatal principal cells (medium spiny neurons) which coexpress both D1 and D2 receptors (Surmeier *et al.*, 1996). While the actions of dopamine in the neostriatum are varied depending on dopamine receptor subtype and cellular phenotype, an important action of dopamine D1 receptors is to

enhance the glutamatergic input arriving from the prefrontal cortex/neocortex (Nicola *et al.*, 2000).

The n. accumbens shell region appears to be part of the extended amygdala and has yet different input–output relationships compared to the n. accumbens core (Heimer, 2003). In fact, the shell region is considered to be a constituent of the extended amygdala involving the n. accumbens shell, the central n. of the amygdala, and the bed n. of the stria terminalis (BNST).

Studies involving the lesioning of dopaminergic terminals in the striatum have revealed interesting relationships between dopamine and serotonin. Namely, neonatal destruction of dopaminergic nerve terminals in the dorsal striatum results in a 5-HT hyperinnervation of the adult striatum as reviewed elsewhere (Kostrzewa *et al.*, 1998).

VII. Dopamine and Serotonin in the Hippocampal Formation

The hippocampal formation includes the dentate gyrus, the four subdivisions of Ammon's horn (CA1/2/3/4), the subiculum, the entorhinal cortex, and the parahippocampal gyrus. Both the rat and cynomolgus monkey have a rich dopaminergic innervation of the entorhinal cortex, subiculum, and CA1 and/or CA3 (Baulac *et al.*, 1986; Samson *et al.*, 1990). Dopaminergic-containing neurons of the VTA project to the ventral hippocampus (Gasbarri *et al.*, 1994; Verney *et al.*, 1985).

All five dopamine receptor subtypes are expressed in the hippocampal formation, though with a differential distribution (Meador-Woodruff *et al.*, 1994). There is also a rich serotonergic innervation of the hippocampal formation from both the dorsal and especially the median raphe (Hensler, 2006). Of the serotonin receptors, the 5-HT_{1A} receptor has been associated with playing important functional roles in the hippocampus (Andrade and Nicoll, 1987; Haddjeri *et al.*, 1998). Alterations in the oscillatory frequencies of the hippocampus have been associated with important behavioral state changes; 5-HT_{1A} receptors in the hippocampus have been implicated in modulating hippocampal oscillations (Gordon *et al.*, 2005). The 5-HT_4 receptor induces a slow excitatory response to 5-HT in the hippocampus which has been compared functionally to 5-HT_{2A} receptor activation in the neocortex (Andrade and Chaput, 1991). In this respect, the 5-HT_4 in addition to 5-HT_{1A} receptors has been implicated as a potential target for cognitive-enhancing therapies for schizophrenic patients (Roth *et al.*, 2004). The 5-HT_6 receptor, localized in the hippocampus, striatum, cerebral cortex, and modulates cholinergic neurotransmission, is another target for cognitive enhancement by selective antagonists (Roth *et al.*, 2004).

The subiculum is important as a site of routing incoming activity to the hippocampus, prior to processing in the dentate gyrus and Ammon's horn. For example, these regions receive afferents from the reunions n. and the anterior tier nuclei of the thalamus (Berendse and Groenewegen, 1991; van Groen and Wyss, 1995; Van Groen *et al.*, 1999). The ventral hippocampus, which has important anatomical relationships to the prefrontal cortex and n. accumbens, is of special interest with respect to neurodevelopmental disturbances of cortico-limbic circuits in schizophrenia. The hippocampus provides a monosynaptic input to the prefrontal cortex via the subiculum and temporal aspect of CA1 (Jay *et al.*, 1989; Laroche *et al.*, 1990; Wyss *et al.*, 1980). There does not appear to be a specific relationship with hippocampal afferents to the medial prefrontal cortex similar to the thalamocortical afferents from the medial dorsal n. (Carr and Sesack, 1996; Kuroda *et al.*, 1996). The subiculum also provides a massive input to the n. accumbens that is necessary for accumbal neurons to enter a depolarized, "up" state (O'Donnell and Grace, 1995). Dopamine D1-like and probably D2 receptors play at least a permissive role in the locomotor activation and increase in n. accumbens extracellular dopamine induced by local infusion of NMDA into the ventral hippocampus (Zornoza *et al.*, 2005).

VIII. Dopamine and Serotonin in the Prefrontal Cortex/Neocortex

Dopamine and serotonin are two of the aminergic neurotransmitters (along with norepinephrine and histamine) that are constituents of the ascending arousal system from the brain stem which also includes the reticular activating system. As such, an assessment of the role for dopamine and serotonin for cortical function must include their role in the ascending arousal system and the modular nature of the prefrontal cortex/neocortex (Grillner *et al.*, 2005; Silberberg *et al.*, 2005). The thick, tufted layer V pyramidal cells are the principal output cell for the prefrontal cortex/neocortex with projections to the thalamus, striatum, amygdala, brain stem, and spinal cord (Deschenes *et al.*, 1994). These layer V pyramidal cells provide the "driving" input to the midline and intralaminar thalamic nuclei discussed above (Sherman and Guillery, 1998). Untufted layer V pyramidal cells that do not extend tufts of dendrites up into layer I of the prefrontal cortex/neocortex project to the contralateral hemispheres. A population of layer VI corticothalamic pyramidal cells project to the first-order thalamic nuclei as a "modulatory" input. Thus, understanding relative dopaminergic or serotonergic control of layer V versus layer VI pyramidal cells has important implications for controlling or modulating different types of thalamocortical pathways.

Different subpopulations of cortical pyramidal cells also project to the striatum (Groenewegen *et al.*, 1999). For the rat ventral prelimbic region of the prefrontal cortex, for example, deep layer V pyramidal cells project to the dorsomedial portion of the shell and "patches" (see below) of the n. accumbens core. In contrast, layer III and superficial layer VI pyramidal neurons send fibers to the "matrix" compartment of the n. accumbens core.

Most of the remaining 20% of PFC/neocortical neurons are GABAergic interneurons which form a variety of classes based on nature of principal efferent contact (interneuron vs pyramidal cell), portion of the axonal/soma/dendritic surface that is contacted by the interneuron, nature of calcium-binding protein expressed by the cell, nature of peptide neurotransmitters colocalized to the interneuron (e.g., substance P, neurokinin B, CCK), and the electrophysiological phenotype of the interneuron. The critical importance of interneurons in sculpting and modulating oscillations of the cortex is highlighted for schizophrenia given the importance of some oscillations such as those in the gamma range as a substrate for higher cortical function (Symond *et al.*, 2005). In addition to the role of interneurons in shaping oscillations, the observations that interneurons appear to be involved in the pathophysiology of schizophrenia makes understanding the role of dopamine and 5-HT for interneuron function a critical question (Levitt *et al.*, 2004).

Differences exist for the serotonergic and dopaminergic projections to the prefrontal cortex and neocortex. First, serotonergic terminals are more widely and evenly distributed in different regions of the prefrontal cortex and neocortex (Tork, 1990). This is in contrast to the dopaminergic system which is limited largely to the prefrontal cortex in the rodent. The primate, however, has an expanded regional dopamine distribution outside of the prefrontal cortex (Berger *et al.*, 1991). Second, while both serotonergic and dopaminergic projections do differ in having distinct laminar distributions, they may even differ from region to region or even in different primate species (Crino *et al.*, 1993). It is the deep projections of dopaminergic cells to layer VI of the prefrontal cortex which appear to be diminished in schizophrenic patients (Akil *et al.*, 1999).

One area of similarity between serotonergic and dopaminergic neurotransmission in the prefrontal cortex and neocortex is both the absence (volume transmission) and the presence (synaptic transmission) of synaptic specializations associated neurotransmitter-releasing monoaminergic varicosities. Estimates for the frequency of synaptic specializations opposite to serotonin varicosities ranges from 28% to 90% in the rat prefrontal cortex or neocortex (Papadopoulos *et al.*, 1987; Seguela *et al.*, 1989). In contrast, very few synaptic specializations were observed opposing serotonergic varicosities in the primate motor-sensory cortex (DeFelipe and Jones, 1988). These observations of synaptic versus volume transmission may be of particular importance with regard to activation of particular

monoaminergic neurotransmitters on different cellular compartments when neurotransmitter release, reuptake, or metabolism is altered.

Interneurons have emerged as a significant target of 5-HT-containing varicosities for direct synaptic neurotransmission in the primate (Smiley and Goldman-Rakic, 1996), where only ~8% of the postsynaptic shafts opposing serotonin axons were identified as pyramidal cell dendrites. 5-HT_{2A} receptors have been localized to large- and medium-sized parvalbumin and calbindin-containing interneurons that would likely target the perisomatic region of pyramidal cells (Jakab and Goldman-Rakic, 1998, 2000). This is striking since the evidence suggests that most, if not all, cortical pyramidal cells contain 5-HT_{2A} receptors. In contrast, 5-HT_3 receptors are present solely in classes of interneurons that target the dendritic field of pyramidal cells (Jakab and Goldman-Rakic, 2000; Morales *et al.*, 1996).

One effect of activating 5-HT_{2A} receptor-containing interneurons in the cortex is to increase spontaneous inhibitory postsynaptic currents (IPSCs) recorded from pyramidal cells through layers II–VI. In layer V pyramidal cells, activation of 5-HT_{2A} receptors results in depolarization by an apparent closure of potassium channels and may also increase a persistent sodium current (Aghajanian and Marek, 1997; Araneda and Andrade, 1991; Tanaka and North, 1993). Another effect that is largely restricted to the major output cell of the prefrontal cortex and neocortex is an enhancement of spontaneous EPSCs which appears to involve an induction of glutamate release from thalamocortical afferents (layers I and Va) onto the apical dendritic field of layer V pyramidal cells (Aghajanian and Marek, 1997; Lambe *et al.*, 2000; Marek *et al.*, 2001). In contrast to these direct excitatory effects on different cellular compartments by activation of 5-HT_{2A} receptor, it should be kept in mind that the predominant effects of 5-HT in the cortex during electrically evoked potentials is a suppression of activity that appears to washout quickly when probed using *in vitro* slice preparations. This inhibitory effect on glutamatergic transmission is due at least in part to activation of 5-HT_{1B} receptors (Aghajanian and Marek, 1999; Read *et al.*, 1994; Tanaka and North, 1993).

In contrast to the relationship of serotonergic varicosities to synaptic specializations in interneurons, the major relationship for dopaminergic varicosities to synaptic specializations appears to be on pyramidal cell dendrites (Goldman-Rakic *et al.*, 1989; Smiley and Goldman-Rakic, 1993). While some of the dopamine D1 receptors in the human and monkey prefrontal cortex appear to be in association with synaptic specializations on dendritic spines opposite of presumed glutamatergic terminals, many dopamine D1 receptors may also have an extrasynaptic localization in pyramidal cells (Smiley *et al.*, 1994). In contrast to dopamine D1 and D5 receptors, the dopamine D4 receptor has been localized to GABAergic interneurons (Mrzljak *et al.*, 1996). The electrophysiological effects of dopamine

in the prefrontal cortex have been reviewed elsewhere (Seamans and Yang, 2004).

Groundbreaking studies emphasizing the importance of prefrontal cortical dopamine in modulating spatial working memory was the finding that depletion of dopamine in the rat or primate mPFC resulted in deficits approximately as robust as prefrontal cortical lesions (Brozoski *et al.*, 1979; Simon *et al.*, 1980). A number of studies since that time especially by Arnsten, Goldman-Rakic, and colleagues demonstrated that activation of dopamine D1 receptors facilitated prefrontal cortical executive function in the primate and rodent. Similarly, activation of dopamine D1 receptors in the mPFC increases the accuracy of rats performing on a five choice serial reaction time test (5-CSRTT) which measures attention (Granon *et al.*, 2000). An inverted "U" relationship between D1 receptor stimulation and PFC function exists (Zahrt *et al.*, 1997). Furthermore, enhanced dopaminergic function also appears to play a role in the disruption of working memory in the dorsolateral prefrontal cortex induced by stress (Arnsten and Goldman-Rakic, 1998).

Evidence also supports the hypothesis that activation and blockade of 5-HT_{2A} receptors impair and enhance, respectively, working memory tasks for which the DLPFC is a critical component. The hallucinogen LSD impairs delayed response and delayed alternation tasks in the rhesus monkey in a fashion consistent with prefrontal cortical lesions (Frederick *et al.*, 1997; Jarvik and Chorover, 1960). 5-HT_{2A} receptor activation appears to improve the tuning of PFC pyramidal cells and interneurons in primates performing a well-learned delayed response task. It was suggested that activation of 5-HT_{2A} receptors would adversely impact cognition with more demanding tasks (Williams *et al.*, 2002). Accordingly, the selective 5-HT_{2A} receptor antagonist EMD 281014 enhanced the performance of both young and aged rhesus monkeys on a delayed response task (Terry *et al.*, 2005). However, the effects of LSD appear to be more prominent on time estimation and motivation, than on short-term memory and attention (Frederick *et al.*, 1997). Similarly, hallucinogens that selectively activate the 5-HT_2 family of receptors have also been found to have profound effects on social interaction, especially affiliative behavior (Schlemmer and Davis, 1986).

Serotonin appears to play a role in a number of aspects of impulsivity as known from rodent studies and a review of the primate and human literature where 5-HT levels were decreased either by global lesion of serotonergic neurons or dietary manipulation (Winstanley *et al.*, 2004a). A number of studies using systemic administration of phenethylamine hallucinogenic drugs which activate the 5-HT_2 family of receptors or 5-HT receptor antagonists with at a 20- to 80-fold selectivity for 5-HT_{2A} versus 5-HT_{2C} receptors suggest that activation of 5-HT_{2A} impairs impulsivity as reflected in an increased premature responding on the 5-CSRTT (Carli and Samanin, 1992; Koskinen *et al.*, 2000; Passetti *et al.*, 2003; Winstanley *et al.*, 2004b). A local effect in the mPFC for 5-HT_{2A} receptors

on modulating impulsivity in this task is supported both by the ability of systemic administration of the selective 5-HT_{2A} receptor antagonist M100907 to prevent attentional impairment induced by local NMDA receptor blockade or for local infusion of M100907 itself to decrease premature responding and omissions (Carli *et al.*, 2004; Winstanley *et al.*, 2003).

Opposing influences of different 5-HT receptor subtypes have also been found using the 5-CSRTT. For example, in contrast to the improvement in impulsivity with systemic or local administration of a 5-HT_{2A} receptor antagonist, systemic administration of a 5-HT_{2C} receptor antagonist SB 242084 increased impulsivity (Winstanley *et al.*, 2004b). Analogous data has been produced where a selective 5-HT_{2A} receptor antagonist attenuated the increase in premature responding induced by NMDA receptor blockade whereas the 5-HT_{2C} receptor antagonist SB 242084 was without an effect (Higgins *et al.*, 2003). However, a 5-$HT_{2B/2C}$ receptor antagonist did not share this effect with SB 242084 (Talpos *et al.*, 2006). Within the prefrontal cortex, local activation of 5-HT_{1A} receptors also improves visuospatial attention and decreases impulsivity, though with some differences in comparison to local blockade of 5-HT_{2A} receptors (Winstanley *et al.*, 2003). This observation would be consistent with the hypothesis that activation of 5-HT_{1A} and blockade of 5-HT_{2A} receptors could have therapeutic effects for cognitive impairment of schizophrenic patients (Roth *et al.*, 2004). In contrast to the effects of 5-HT_{2A} receptor blockade, a 5-HT_6 receptor antagonist had no effect on impulsivity (Talpos *et al.*, 2006). While these results with 5-HT_{2A} receptor blockade show a consistent effect on impulsivity as measured by premature responding on the 5-CSRTT, it should be noted that 5-HT_{2A} receptor antagonists did not counter impulsivity observed for choice procedures between a small immediate reward versus a larger, delayed reward (Talpos *et al.*, 2006; Winstanley *et al.*, 2004a).

IX. Animal Models

Two principal unmet medical needs in treating schizophrenic patients is improving negative symptoms and treating the cognitive impairment associated with schizophrenia (CIAS). The probability that a novel treatment would treat both the positive symptoms and cognitive impairment/negative symptoms in the near future seems remote. The MATRICS and TURNS initiative involving academia, industry, and the FDA assumes that new treatments targeting cognitive deficits will be added to current antipsychotic drugs (Geyer and Tamminga, 2004). A critical feature in moving toward these new treatment goals is a deeper appreciation of the neural substrates involved with respect to macrocircuitry relevant for schizophrenia (thalamocortical-striatal-hippocampal-brain stem).

An important task of future research is understanding the degree in which different cognitive enhancement strategies will be compatible with current antipsychotic drugs (Floresco *et al.*, 2006).

The neonatal excitotoxic hippocampal lesion (ventral hippocampus and ventral subiculum) is an animal model with evidence of altered dopaminergic activity in addition to decreased social behavior, impaired working memory, and enhanced sensitivity to drugs of abuse (Lipska, 2004). A developmental link with this model is emphasized by the ability of excitotoxic lesions of the mPFC to block certain behaviors in this syndrome such as hyperlocomotion to novelty and amphetamine (Lipska *et al.*, 1998). Furthermore, even reversible inactivation during the neonatal period leads to some of the characteristic dopamine- and glutamate-mediated changes in pharmacological sensitivity (Lipska, 2004). An additional key feature with this model is an enhanced sensitivity to the effects of NMDA antagonists which imparts additional clinical face validity.

Another developmental model for schizophrenia involves administering the methylating agent methylazoxymethanol acetate (MAM) on embryonic day 17. When the rats are tested as adults, they have a number of neuroanatomical features consistent with the neuropathology of schizophrenia such as size reduction in the mediodorsal thalamus, hippocampus, and prefrontal cortex and decreased neuron density without neuron loss in the prefrontal cortex (Moore *et al.*, 2006). These rats also exhibit a disruption of synaptically driven bistable membrane potentials in both the prefrontal cortex and the ventral striatum (Lavin *et al.*, 2005). A remarkable cognitive change in these rats is a deficit in reversal learning. With respect to the dopaminergic system in the prefrontal cortex, these rats exhibit a hypofunctional response to topical dopamine while the deep pyramidal cells are more sensitive to VTA stimulation. At a behavioral level, adult (but not adolescent) rats exhibit greater sensitivity to the locomotor activating effects of amphetamine (Moore *et al.*, 2006).

Since increases in striatal dopamine D2 receptors have been implicated in the pathophysiology of first-break and medicated schizophrenic patients, mice have been generated with a reversible increase level of striatal D2 receptors (Kellendonk *et al.*, 2006). These mice have cognitive deficits in the sphere of attentional set-shifting and also have decreased dopamine turnover in the prefrontal cortex that are consistent with altered function in the prefrontal cortex.

Another recent animal model for schizophrenia is a mouse strain with a dramatically reduced level of the obligate NMDA NR1 receptor subunit. These mice display reduced locomotor habituation to a novel environment, increased stereotypic activity, deficits in social interaction, and reduced prepulse inhibition of acoustic startle (Duncan *et al.*, 2004; Mohn *et al.*, 1999). Another characteristic of these mice bearing some similarity with schizophrenic patients is an increased motor stereotypy and reduced fos expression in the medial prefrontal and cingulate cortex in response to amphetamine (Miyamoto *et al.*, 2004).

X. Conclusions

A fundamental question in understanding the place of dopamine and serotonin interactions in treating schizophrenic patients will be the extent and ease that cognitive impairment and negative symptoms/deficit state can be treated by layering new therapeutics on a base of atypical antipsychotic drugs. Understanding the interactions of dopamine and serotonin with respect to modulating amino acid neurotransmission in macrocircuits involving the prefrontal cortex, the striatum, the thalamus, the hippocampus, and the brain stem with the ascending reticular activating system is a critical feature. Cognitive enhancement by modulating dopaminergic D1 receptor neurotransmission remains a relatively untapped direction. Cognitive enhancement with 5-HT_{1A} partial agonists, 5-HT_{2A} antagonists, 5-HT_4 partial agonists, and 5-HT_6 antagonists would appear to address these remaining unmet medical needs in schizophrenia. One region of the brain which would appear to require a greater emphasis would be the thalamus given the chemical heterogeneity of this anatomical structure and the relatively understudied components such as the midline and intralaminar thalamic nuclei, which appear to provide a crucial anatomical node that complements other thalamic systems which are easier to study such as the mediodorsal nucleus.

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CHOLINERGIC CIRCUITS AND SIGNALING IN THE PATHOPHYSIOLOGY OF SCHIZOPHRENIA

Joshua A. Berman,* David A. Talmage,† and Lorna W. Role*,‡

*Department of Psychiatry, Columbia University College of Physicians and Surgeons
New York State Psychiatric Institute, New York, New York 10032, USA
†Institute of Human Nutrition, Columbia University College of Physicians and Surgeons
New York, New York 10032, USA
‡Department of Cell Biology in Pathology, Columbia University College of Physicians and
Surgeons, New York, New York 10032, USA

INTERNATIONAL REVIEW OF NEUROBIOLOGY, VOL. 78
DOI: 10.1016/S0074-7742(06)78007-2

0074-7742/07 $35.00

Central cholinergic signaling has long been associated with aspects of memory, motivation, and mood, each affected functions in neuropsychiatric disorders such as schizophrenia. In this chapter, we review evidence related to the core hypothesis that dysregulation of central cholinergic signaling contributes to the pathophysiology of schizophrenia. Although central cholinergic circuits are resistant to simplification—particularly when one tries to parse the contributions of various classes of cholinergic receptors to disease related phenomena—the potential role of ACh signaling in Schizophrenia pathophysiology deserves careful consideration for prospective therapeutics. The established role of cholinergic circuits in attentional tuning is considered along with recent work on how the patterning of cholinergic activity may modulate corticostriatal circuits affected in schizophrenia.

I. Introduction

Cholinergic innervation of cortical and striatal brain areas is extensive and diffuse, as are both pre- and postsynaptic targets for acetylcholine (ACh) interaction. Receptors for ACh (AChRs) come in two broad classes—ionotropic (nicotinic) and metabotropic (muscarinic)—each class having multiple subtypes with both opposing and synergistic actions. Activation of these receptors regulates neuronal excitability by interaction with pre- and postsynaptically localized ACh-binding sites. ACh can act as a tonic, diffuse signal, modulating the release of ACh and other transmitters, including dopamine, glutamate, and GABA. Alternatively, ACh can exert its effects via highly localized and directed interactions with neuronal AChRs to increase or decrease neuronal firing.

The complexity of CNS cholinergic circuits and signaling mechanisms produces a system in which origins and end results may be easier to appreciate than intervening steps. It is clear that ACh, released from the cholinergic inputs of the basal forebrain, striatal, and the pontomesencephalic (PM) areas, plays an important role in supporting neurocognitive and motivational functions of the prefrontal cortical, hippocampal, and ventral tegmental projections to the striatum (for reviews see Cragg, 2006; Gotti and Clementi, 2004; chapter by Martin and Freedman, this volume; Mesulam, 2004; Sarter *et al.*, 2005; Smythies, 2005; Wonnacott *et al.*, 2005). In addition, there is considerable evidence that events which reduce the amount of ACh at cholinergic targets may contribute to functional deficits—including deficits related to schizophrenia (Hyde and Crook, 2001; Sarter *et al.*, 2005; chapter by Martin and Freedman, this volume). But considerable confusion sets in when one tries to extract exactly *how* the intervening steps, with activation of muscarinic and/or nicotinic receptors and consequent changes in downstream circuits, are integrated to elicit the broad spectrum of effects modulated by cholinergic signaling.

Some of the confusion arises from attempts to reconcile the varying "anticholinergic" properties of antipsychotic medications with data on the effects of muscarinic agonists per se. Further confusion arises from the fact that commonly used cholinergic ligands may be less specific in their binding properties than previously thought: indeed, some compounds traditionally considered selective muscarinic antagonists may function as partial agonists or antagonists of other ACh (nicotinic and muscarinic) receptor subtypes. Finally, schemes that overemphasize the role of a particular ACh-signaling pathway to the exclusion of others, rather than viewing the function of cholinergic circuits as the result of the summation of actions of ACh at all of its receptors, may do more to confuse than enlighten. Anatomical and functional data underscore the interaction of cholinergic circuits with other neurotransmitter systems (Smiley *et al.*, 1999). Indeed, interaction of ACh with its full panoply of receptor sites elicits substantive changes in the synaptic transmission of dopamine, glutamate, serotonin, and GABA in a variety of brain regions.

We will first provide a thumbnail sketch of cholinergic circuits and then examine how they are involved in functions relevant to schizophrenia with focus on interactions with dopamine- and glutamate-mediated signaling. We will then review developmental/genetic, pathological, and pharmacological evidence for potential cholinergic contributions to schizophrenia. Although cholinergic signaling may not be the major site of circuit dysregulation underlying the etiology of schizophrenia, more knowledgeable manipulation of cholinergic systems may provide an untapped reservoir of considerable therapeutic potential in the treatment of the positive and negative symptoms of this complex disease.

II. ACh in Brain Regions Implicated in Schizophrenia

Central cholinergic circuits participate in aspects of memory formation, motivational and volitional behaviors, and affect. Each of these functions is altered in neuropsychiatric disorders, including schizophrenia. Cholinergic neurons in the CNS make up for any apparent deficit in numbers by projecting to a broad swath of cerebral cortical mantle, select portions of the temporal lobe, and by their profuse axonal arborizations throughout the corpus striatum. The schematic diagram presented in Fig. 1 attempts to bring some order to the cholinergic chaos by corralling the diverse targets of ACh innervation into a manageable subset of brain regions strongly implicated in schizophrenia. Our focus is represented in primary colors: red for cholinergic neuronal groups and a subset of their projections, and yellow for the chosen cholinoceptive targets—brain regions that have been examined in detail in recent circuit analyses and that will be the focus of this chapter. Obviously this degree of simplification endangers the generality of our

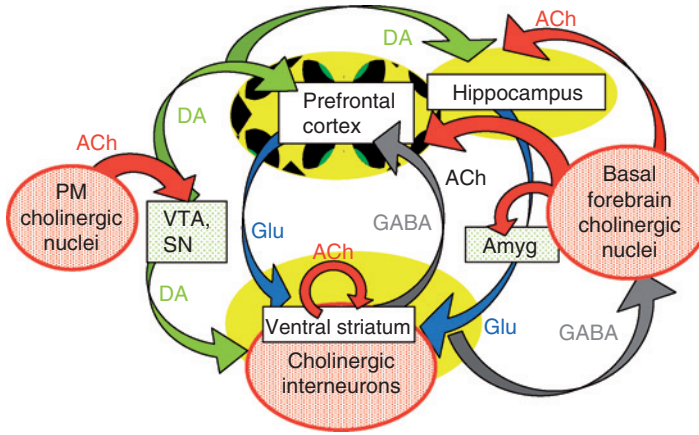

FIG. 1. Schematic diagram of cholinergic circuits (in red) and their projections within a subset of key brain regions affected in SZ. Cholinergic inputs to prefrontal cortex and hippocampus arise primarily from the basal forebrain group, including the septal cholinergic neurons, the nbM, the preoptic and diagonal band nuclei. Other contributors to the forebrain ACh group are neurons within the substantia innonimata and ventral pallidum. The second major subgroup of ACh-containing neurons, the pontomesencephalic (PM) cholinergic neurons, provides input to brainstem aminergic nuclei (e.g., VTA, SN, and raphe). Cholinergic interneurons intrinsic to the basal ganglia are thought to modulate the relative impact of glutamatergic, dopaminergic, and GABAergic circuits within the ventral striatum. Potential mechanisms of cholinergic regulation of neuronal excitability in prefrontal cortex and hippocampus are also discussed in the text.

considerations—for example, there is little discussion of cholinergic signaling in amygdala, or in cingulate or somatosensory cortex—areas of study that have contributed important progress to our understanding of central cholinergic coding. Such omissions are not intended to infer relative impact on the field, but rather reflect the limits of time, space, and comprehension of the authors.

Overall, there are three major groups of cholinergic neurons and interneurons within the primate brain. Cholinergic inputs to prefrontal cortex and hippocampus arise primarily from the basal forebrain group, which includes the medial septal cholinergic neurons, the nucleus basalis of Meynert (nbM) and the preoptic and diagonal band nuclei (Fig. 1, right). Other contributors to the forebrain ACh groups are neurons within the substantia innominata and ventral pallidum. The relative contribution of cholinergic versus noncholinergic neurons to each of the basal forebrain nuclei ranges from <5% to 90% in human brain, with the nbM as the highest density forebrain cholinergic nucleus (Mesulam *et al.*, 1984; reviewed in Hyde and Crook, 2001). Other major targets of the basal forebrain groups include the amygdala, olfactory bulb, and hypothalamus (Woolf, 1991).

The second major subgroup of ACh-containing neurons (Fig. 1, left), the PM cholinergic neurons, provides input to brainstem aminergic nuclei (e.g., VTA,

SN, raphe) as well as to the cerebellum, thalamus, and hypothalamus (Woolf, 1991). In addition, the PM group of neurons project to the basal forebrain cholinergic neurons, thereby coordinating central cholinergic modulation of brainstem, midbrain, and forebrain circuits.

The final major group of cholinergic neurons consists of the ACh interneurons that are intrinsic to the basal ganglia. These intrastriatal neurons modulate the relative impact of multiple glutamatergic and dopaminergic circuits on the medium spiny GABAergic projection neurons of the striatum: our focus will be on the role of cholinergic circuits in the regulation of ventral striatal signaling.

A. Cholinergic Pathways Within the Ventral Striatum

Cholinergic neurons within the striatum are typically large, aspiny neurons that comprise 1–5% of the striatal interneurons, varying somewhat with species and consideration of dorsal versus ventral regions. The extensive arborizations of the striatal cholinergic interneurons throughout the corpus striatum provide a tonic level of ACh release, with the ultimate concentration of extracellular ACh being set (and reset) by the interplay of local ACh release and the activity of the omnipresent acetylcholinesterase (AChE).

Striatal cholinergic neurons are characterized by their tonic activation profile, and periods of relatively low activity (referred to as "pauses") can be associated with salience and prediction of reward (Cragg, 2006; Graybiel *et al.*, 1994). The phasic lulls and peaks of the striatal cholinergic activity, which alters local ACh and choline concentrations, are influenced by corticostriatal projections from hippocampal subicular and prefrontal glutamatergic neurons (Fig. 1, in blue), as well as from amygdala, cingulate, and other cerebral and temporal cortices (not shown). In the rodent, the bulk of the hippocampal projections to the nucleus accumbens arise from ventral rather than dorsal hippocampal areas analogous to the more anterior portions of the primate hippocampus.

Dopaminergic inputs to the ventral striatum arise from the ventral tegmentum (VTA) and substantia nigra (SN), which themselves are recipients of cholinergic projections from PM neurons. Activation of a variety of pre- and postsynaptic dopamine receptors strongly regulates the release of ACh and the excitability of striatal cholinergic interneurons (Maurice *et al.*, 2004; Wang *et al.*, 2006). In addition, local circuits of opiate peptide and GABAergic neurons influence the net levels of striatal cholinergic tone.

Even this, admittedly limited, summary of key regulators of the spatial and temporal profile of ACh-mediated signaling in striatum reveals considerable complexity. Nevertheless, recent progress in dissecting the interaction of cholinergic circuits with dopaminergic and glutamatergic inputs to the striatum inspires considerable hope that we may be approaching a *bona fide* understanding

of how ACh works at least in *one* region that is known to effect in schizophrenia and that is particularly high in ACh tone (see below and Cragg, 2006; Calabresi *et al.*, 2000; Wilson, 2006; Wonnacott, 2005 for reviews; Wang *et al.*, 2006).

B. Cholinergic Projections in Prefrontal Cortex and Hippocampus

The principal source of cholinergic input to the PFC and hippocampus is from the basal forebrain nuclei, with particularly strong contributions from the medial septum in rodent and from the nbM in human brain. The primate prefrontal cortex receives a fairly homogeneous cholinergic input with the highest density of cholinergic marker-positive fibers in layers I, II, and V (Lewis, 1990; Smiley *et al.*, 1997). Cholinergic axons within the cerebral cortex of human brain are studded with numerous *en passant* swellings that serial EM reveals as primarily asymmetric type synapses. Close appositions of cholinergic synaptic profiles in cortex (Mesulam, 1999, 2004; Smiley *et al.*, 1997) as well as the prevalence of cholinergic marker-positive swellings in the vicinity of pyramidal and nonpyramidal neurons in PFC and hippocampus is consistent with proposed modulatory effects of ACh on both excitatory and inhibitory cortical circuits (Mansvelder *et al.*, 2006). Likewise, evidence has accrued that the release of ACh per se is likely subject to cholinergic, as well as dopaminergic, synaptic tuning in both PFC and hippocampus (DeBoer *et al.*, 1996; Moore *et al.*, 1999).

III. Physiology of ACh Circuits and Signaling in Brain Regions Implicated in Schizophrenia Pathology

A. ACh Receptors in the CNS

When clinicians and patients contemplate the "anticholinergic" side effects of various drugs, they often focus on the diverse and distressing array of peripheral autonomic cholinergic actions, including alterations in gastrointestinal function, nausea, and changes in appetite. In fact, the binding sites with which cholinergic drugs interact in the CNS are just as diverse as those in the periphery and often more accessible than expected, despite the blood–brain barrier. Any careful deliberation on ACh-binding sites in the CNS must include ACh-degradative, synthetic, and transporter proteins, as well as the multimembered muscarinic and nicotinic receptor subtypes (for reviews see Calabresi *et al.*, 2000; Cobb and Davies, 2005; Gotti and Clementi, 2004; Mansvelder *et al.*, 2006; Newhouse *et al.*, 2004; Sarter *et al.*, 2005). Pharmacological agents originally identified for their

activity as AChE inhibitors (such as physostigmine and galantamine) are now known to act as partial agonists or antagonists of specific subtypes of CNS nicotinic AChRs (nAChRs). The oldest and most established "antimuscarinic" agent, atropine, blocks multiple classes of nAChRs at submicromolar concentrations—well within the clinically relevant and experimentally typical range of doses (Zwart *et al.*, 1999). Carbamylcholine is more than a muscarinic agonist—it gates deliciously long openings of nAChRs. Finally, the activation of different types of presynaptic nicotinic *and* muscarinic receptors can facilitate or depress the release of ACh itself (see below).

Awareness of the complexities in the number and pharmacodiversity of ACh-binding partners in the CNS is essential to evaluating the past and present literature on ACh circuits and signaling. Humbling though this may be, we are actually well positioned to do so: the last 20 years have yielded impressive advances in understanding the differential regulation, expression, targeting, and function of the many muscarinic (at least 5 genes, so far) and nicotinic (11 subunit genes) receptors (see below). With this knowledge in hand, we need to reassess the effects of the pharmaceuticals we have and work toward the development of agents that more selectively manipulate the synthesis, release, and binding(s) of ACh.

A quick primer then, on the most important of ACh-binding sites, from information largely extracted from the following reviews: Calabresi *et al.* (2000); Gotti and Clementi (2004); Laviolette and van der Kooy (2004); MacDermott *et al.* (1999); Mansvelder *et al.* (2006); Sarter and Parikh (2005); Smythies (2005); Wonnacot *et al.* (2005).

1. *Choline acetyltransferase (ChAT):* This enzyme is responsible for ACh synthesis. The regulation of ChAT gene expression in the CNS is thought to be coordinated with that of vAChT, by virtue of a common "cholinergic locus" promoter. However, the distribution of these two proteins between somatodendritic and axonal domains may be regulated independently.

2. *ACh esterase (AChE):* This binds ACh with micromolar affinity and is considered the principal degradative activity for ACh. AChE is one of the fastest turnover rate enzymes identified and is located primarily at intraneuronal and extracellular sites. Despite its preeminence as "the AChE," recent work deleting AChE-encoding genes revealed that butyrylcholinesterase (BuChE) activity, which is associated with glial cells rather than neurons, can maintain grossly normal ACh balance. So BuChE is another ACh-binding partner to bear in mind.

3. *The vesicular ACh transporter (vAChT):* This binds ACh with submicromolar affinity and translocates it into vesicular compartments within cholinergic neurons.

4. *Muscarinic (metabotropic) AChRs:* At least five genes are identified to date (M1–M5); M1, M2, and M4 subtypes predominate in the CNS. These ACh-binding proteins are coupled to a variety of G-proteins resulting in the

activation or inhibition of an even wider variety of enzymatic and ion channel targets. Note that in the CNS, only a subset of the muscarinic-binding sites are postsynaptic; other subtypes of muscarinic receptors are targeted to axonal/presynaptic sites where they modulate the release of glutamate, dopamine, and ACh, among other key players.

5. *Nicotinic (ionotropic) AChRs:* Twelve subunit genes ($\alpha 2$–$\alpha 10$; $\beta 2$–$\beta 4$) encode a group of proteins that are faintly related to—and pharmacologically very distinct from—the renowned muscle-type nicotinic receptors. Drugs that interact with subtypes of neuronal nicotinic receptors (e.g., nicotine, hexamethonium) barely touch the muscle receptor and vice versa. Also important to note is that in the CNS, nAChRs, just like muscarinic receptors, are targeted to *pre-* as well as postsynaptic locations. In fact, the role of presynaptic nicotinic receptors as modulators of dopamine, glutamate, GABA, serotonin, and ACh release is so prevalent in the CNS that their contribution as postsynaptic receptors is often overlooked (but see Frazier *et al.*, 1998; Jones and Yakel, 1997)!

In sum, a circumspect evaluation of how the dysregulation of cholinergic circuits may be involved in the pathophysiology requires recognition that ACh targets are many, perhaps not as pharmacologically distinct as previously considered and at pre-, post-, and perisynaptic locations. Viewing the function of cholinergic circuits as the result of the summation of actions of ACh at all of its receptors, although initially daunting, may resolve some apparent conflicts in the literature and guide the way to new therapeutic approaches (for reviews see Calabresi *et al.*, 2000; Gotti and Clementi, 2004; Laviolette and van der Kooy, 2004; MacDermott *et al.*, 1999; Mansvelder *et al.*, 2006; Sarter and Parikh, 2005; Sarter *et al.*, 2005; Smythies, 2005; Wonnacot *et al.*, 2005).

B. Physiology of ACh Circuits in Striatum

The striatum is established stomping grounds for fans of central cholinergic circuits and ACh signaling. Although the numbers of cholinergic neurons in the striatum are small, they are the foremost, if not the exclusive, source of the high-pack cholinergic inputs in mammalian striatum. As discussed above, striatal cholinergic neurons are characterized by their large size, aspiny appearance, and tonic activation profile (hence the names ASpN and TANS neurons; Fig. 2). Changes in the activity profile of striatal TANS, referred to as "pauses," are thought to arise in part from the slowing of autonomous pacemaker activity and in part to local changes in dopamine, glutamate, and GABA signaling (Cragg, 2006; Maurice *et al.*, 2004; Wang *et al.*, 2006). The association between changes in striatal cholinergic "tone" and salience/reward prediction has continued to stoke the fire of physiologists' interests in the workings of striatal ACh circuits

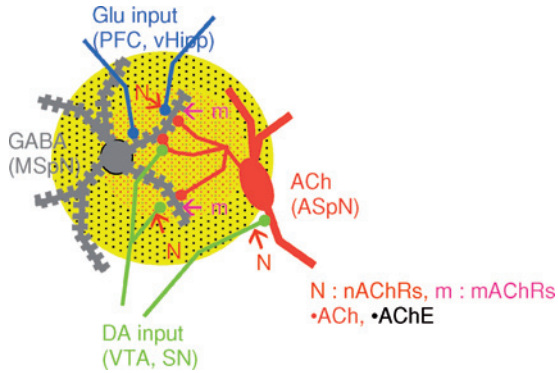

FIG. 2. Schematic diagram of an aspiny cholinergic neuron (ASpN) and its projections to convergent sites of glutamatergic and dopaminergic input on striatal GABAergic medium spiny projection neurons (MSpN). Changes in local [ACh], from pauses in the firing of these TANS, modulate the net output of the striatum by interactions with ACh receptors and binding sites in pre-, post-, and perisynaptic compartments.

(see Cragg, 2006 for review; Apicella, 2002; Maurice *et al.*, 2004; Wang *et al.*, 2006 for recent highlights).

Perhaps the best studied, although still mechanistically mysterious, role of cholinergic circuits in striatum is in their reciprocal interactions with dopaminergic inputs from the VTA and SN. Recent studies add new dimensions to prior evidence that ACh acts as a key regulator of striatal output by influencing the activity of GABAergic medium spiny neurons (MSpNs, Fig. 2). The newest twist is that ACh is likely to exert its modulatory control on striatal activity through interaction with *both* pre- and postsynaptic, nicotinic *and* muscarinic receptors (Fig. 2). Presynaptic nicotinic receptors have long been implicated in the regulation of striatal dopamine release, with reports identifying some of the nAChR subtypes involved in aspects of nicotine addiction in staggering detail (for review see Wonnacott *et al.*, 2005; Tapper *et al.*, 2004 for recent highlights). The vague term "regulation" was intentionally employed because one of the points of controversy has been whether dopamine release in striatum is enhanced or depressed by nicotine. It turns out that the answer may be both, depending on the frequency of firing of the dopamine neurons (see Cragg, 2006 for review; Partridge *et al.*, 2002; Rice and Cragg, 2004; Zhang and Sulzer, 2004; Zhou *et al.*, 2001). The effects of dopamine receptor agonists on modulating the release of ACh in striatum (as well as in PFC and in hippocampus, see below) are also well established (DeBoer *et al.*, 1996). But new results reveal that depending on the type and location of the dopaminergic and muscarinic receptors activated, the net effect may be to stably enhance or depress the activity of the GABAergic

MSpNs (Cragg, 2006; Wang *et al.*, 2006; Wilson, 2006;). Indeed, the potential for mutual tuning of TANS and DANS seems more than flexible enough to account for the differences in valence and timing of all of the synaptic changes observed in striatum (Calabresi *et al.*, 2000; Cragg, 2006; Maurice *et al.*, 2004; Wang *et al.*, 2006).

C. Physiology of ACh Circuits in PFC and Hippocampus

The role of cholinergic signaling in aspects of memory and cognition are typically attributed to the broad spectrum of effects that ACh elicits in altering the excitability of prefrontal cortical and hippocampal circuits (for reviews see Albuquerque *et al.*, 1996; Buzsaki, 2002; Levin *et al.*, 2006; Mansvelder *et al.*, 2006; Newhouse *et al.*, 2004; Picciotto, 2003; Role and Berg, 1996; Sacco *et al.*, 2004; Sarter *et al.*, 2005; Smythies, 2005; Wonnacott *et al.*, 2005). Analysis of the pros and cons of the many theories on *how* ACh actually does what it does to regulate synaptic efficacy in these regions, as with striatum, is best served by considering the potential interaction of ACh with each of its five major sets of binding partners: ChAT, AChE, VAChT, muscarinic, and nAChRs (see discussion above).

ACh transmission in cortex and hippocampus likely involves both localized release and tonic or "volume" transmission (Cobb and Davies, 2005; Vizi and Kiss, 1998). Activation of presynaptic ACh receptors modulates the release of glutamate, ACh, and dopamine in PFC and hippocampus (Colgin *et al.*, 2003; Laplante *et al.*, 2004; Lucas-Meunier *et al.*, 2003), enhancing or depressing transmission depending on the flavor(s) of AChRs expressed (Mansvelder *et al.*, 2006; Sarter and Parikh, 2005; Wonnacott *et al.*, 2005). Postsynaptic mAChRs and nAChRs have also been implicated in the modulation of PFC and hippocampal circuits (Cobb and Davies, 2005; Frazier *et al.*, 1998; Ji *et al.*, 2001; Jones and Yakel, 1997).

Perhaps the most important (albeit still controversial in detail) role of ACh circuits in cortex and in hippocampus is in the regulation of theta rhythm oscillatory activity (Buzsaki, 2002; Calabresi *et al.*, 2000; Cobb and Davies, 2005; Hasselmo, 2005; Lee *et al.*, 2005). Theta-frequency band oscillations constitute a prominent network pattern in all mammals, including humans. Theta activity has been proposed to underlie everything from temporal cooperativity of cortical and subcortical networks to coordinate modifications of synaptic connections within cortex and hippocampus per se (Buzsaki, 2002; Calabresi *et al.*, 2000; Cobb and Davies, 2005; Hasselmo, 2005). In any case, there is no doubt that cholinergic circuits, specifically the septal cholinergic projections, play an essential role in theta oscillations, as selective lesion of the ACh synthesizing neurons in the medial septal/diagonal band nuclei abolishes hippocampal theta.

Discrepancies arise in interpretation of studies that manipulate ACh by different pharmacological means—that is, M1-AChR versus AChE antagonists—which imply that other types of muscarinic and/or nicotinic AChRs may be involved (Buzsaki, 2002; Ji *et al.*, 2001).

IV. Developmental and Genetic Deficits in Schizophrenia That May Influence Function and Assembly of Cholinergic Systems

Schizophrenia is widely viewed as a neurodevelopmental disease, resulting from a combination of environmental challenges acting on susceptible genotypes. Significant progress has been made at identifying both relevant environmental and genetic risk factors (Bresnahan *et al.*, 2005; Harrison and Weinberger, 2005), although we have yet to make progress at understanding how these factors interact to dysregulate relevant circuits in the developing brain. The vertebrate forebrain contains relatively few cholinergic neurons, yet this population exerts widespread modulatory control over essentially all striatal–cortical networks.

Experimental studies have shown that during development, the cholinergic system is especially sensitive to environmental insults (e.g., ethanol, lead, organophosphates, tobacco smoke; Eriksson *et al.*, 2001; Reddy *et al.*, 2003; Robinson, 2002; Thomas *et al.*, 2000). These and other insults would certainly have the potential to interact with genetic vulnerabilities affecting brain development to produce deficits which could contribute to disease states.

A. Development of Cholinergic Systems

Forebrain cholinergic neurons arise early in telencephalic development (~E10 in mouse which approximately corresponds to gestational day 40 in humans; Clancy *et al.*, 2001) in the medial ganglionic eminence, a ventricular/subventricular neurogenic zone that appears as a thickening along the ventral/medial wall of the ventricle (Brady *et al.*, 1989; Furusho, 2006; Marin *et al.*, 2000; Olsson *et al.*, 1998; Semba *et al.*, 1988). Presumptive forebrain projection cholinergic neurons migrate from the MGE (and possibly from the anterior entopeduncular/preoptic area) radially to take up locations in basal forebrain nuclei (medial septum, magnocellular nucleus, diagonal band of Broca), whereas the striatal cholinergic interneurons migrate tangentially from the MGE, occupying dispersed sites throughout the striatal plate. Subsequent to this early birth and migration from the MGE, 1–2 weeks pass before these neurons undergo maturation into cholinergic neurons (Aznavour *et al.*, 2005; Berger-Sweeney, 2003; Mechawar and Descarries, 2001). This delay allows time for other populations of predominantly GABAergic neurons to emerge from the MGE and LGE

and occupy their appropriate positions throughout the striatum and cortex for radial population of the neocortex with pyramidal cells and for the proper targeting of axons from the dorsal thalamus to project through the striatal region to innervate cortical structures (Flames *et al.*, 2004; Lopez-Bendito *et al.*, 2006; van Vulpen and van der Kooy, 1998). Some investigators propose that during this period the presumptive cholinergic neurons provide instructive signals that guide the targeting and differentiation of later born striatal populations (Berger-Sweeney, 2003; Hohmann, 2003; Hohmann and Berger-Sweeney, 1998).

During the first two postnatal weeks, the cholinergic interneurons elaborate robust networks of axons locally within the striatum, whereas the forebrain cholinergic neurons elaborate a wide array of axonal projections that target all neocortical regions (prefrontal, sensory, and motor) and the hippocampal formation. As a result these two relatively small populations of cholinergic neurons maximize their ability to interact with striatal–cortical networks. It is likely that the delay between the migration of newly formed cholinergic neurons from neurogenic zones and the elaboration of axonal projections plays a critical role in properly controlling the final wiring of the forebrain cholinergic system.

A number of recent studies using molecular genetic approaches in mice are beginning to clarify the developmental processes that determine the specification of forebrain cholinergic neurons. In particular, the basics of a cholinergic transcription factor code are emerging. A variety of experimental approaches has demonstrated that expression of several transcription factors is important (Mash 1, Olig2, Lmx7, Lmx8) or essential (Nkx2.1) for generating forebrain cholinergic neurons (Bachy and Retaux, 2006; Furusho *et al.*, 2006; Marin *et al.*, 2000; Mori *et al.*, 2004; Zhao *et al.*, 2003). Thus, the combined expression of these factors, and their target genes, probably accounts for much of the intrinsic identity of the cholinergic phenotype. However, these studies have not yet distinguished between factors that determine how a newborn cholinergic neuron migrates from the MGE (radially into basal septal regions or tangentially into the striatum), or whether an individual cholinergic neuron will elaborate a spatially restricted axonal network, as is the case of the striatal interneurons, or a broadly targeted set of cortical projections.

B. Potential Role of Neuregulin 1

Neuregulin 1–ErbB signaling plays multiple critical roles in proper development of the neocortex, guiding both the tangential migration of MGE-derived GABAergic interneurons (Flames *et al.*, 2004) and proper navigation of axonal projections from the dorsal thalamus into the cortex (Lopez-Bendito *et al.*, 2006). Whether neuregulin also guides the migration and/or axon projections of forebrain cholinergic neurons is not known. We have seen apparent decreases

in the numbers of specific populations of forebrain interneurons in adult mice that are heterozygous for an isoform specific, targeted mutation in the neuregulin 1 gene (Wolpowitz *et al.*, 2000; Johnson, Talmage, and Role, Unpublished data).

Maturation and maintenance of cholinergic neurons depends on a number of extracellular signaling molecules. Of particular relevance to our discussion of cholinergic signaling and schizophrenia are nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and cortical steroids. NGF, BDNF, and glucocorticoids regulate the expression of ChAT, enhance the connectivity of, and promote the survival of cholinergic neurons (Fagan *et al.*, 1997; Grosse *et al.*, 2005; Guijarro *et al.*, 2006; Johnston *et al.*, 1987; Mobley *et al.*, 1986; Phillips *et al.*, 2004; Sofroniew *et al.*, 2001; Takahashi, 1998; Takahashi and Goh, 1998; Ward and Hagg, 2000). Reports have demonstrated altered regulation of NGF (Parikh *et al.*, 2003), BDNF (Weickert *et al.*, 2003, 2005), and the HPA axis (Corcoran *et al.*, 2001, 2003) in schizophrenics. Whether these changes alter corticostriatal cholinergic tone remains to be seen.

Beyond general effects on synaptic structures, there is no clear evidence linking the products of identified schizophrenia susceptibility genes with the cholinergic system, with the notable exception of the neuregulin 1 gene. Neuregulin 1 has been linked to schizophrenia in multiple populations, and disease-associated changes in the relative expression of different neuregulin 1 isoforms is seen in the DFPLC and hippocampus. Neuregulin 1 isoforms play important roles in neurodevelopment, in particular in the patterning of the neocortex (Flames *et al.*, 2004; Lopez-Bendito *et al.*, 2006). At present, these latter roles for neuregulin 1 have focused on tangential migration of cortical interneurons and axonal projections from the dorsal thalamus to the neocortex. Given the relative spatial and temporal parallels between these events (GABAergic interneurons originate in the MGE during an overlapping time frame with the striatal cholinergic interneurons) and the reported decreases in the numbers of ventral striatal cholinergic interneurons in postmortem tissue from schizophrenics, it is important that studies of the role of neuregulin 1 in forebrain development be extended to the cholinergic system as well.

A more direct association between neuregulin 1 and cholinergic signaling exists at the level of the expression of the nAChRs. Two families of neuregulin 1 isoforms were identified originally by virtue of their ability to regulate the expression of nAChRs at peripheral synapses (Falls *et al.*, 1993; Yang *et al.*, 1998). Subsequently, a number of investigators have demonstrated that neuregulin also can increase the synaptic expression of α7-containing nAChRs at central synapses (Kawai *et al.*, 2002; Liu *et al.*, 2001), and our laboratories have extended this story by demonstrating that neuregulin 1 signaling also regulates presynaptic expression and targeting of the α7 nAChRs (Role and Talmage, unpublished). These latter studies are particularly intriguing in light of the

well-documented deficits in $\alpha 7$ nAChRs in schizophrenics, the association of this deficit with defects in P50 measures of auditory gating in schizophrenics and their first degree relatives, and the association of $\alpha 7$ subunit gene promoter polymorphisms with these deficits (see chapter by Martin and Freedman, this volume; Leonard *et al.*, 1996, 2002).

V. Clinical and Preclinical Evidence for Deficits in Components of Brain Cholinergic Systems in Schizophrenia

There are numerous examples of deficits in components of brain cholinergic systems that have been linked with schizophrenia. They range from abnormal expression of receptors of various subtypes through decreases in cholinergic neurons in key areas.

A. Deficits in Components of Muscarinic Cholinergic Transmission

Several studies give evidence of alteration of muscarinic ACh receptors in the brains of schizophrenics. The majority of evidence in this realm points to decrements in binding suggestive of a decrease in available m1 muscarinic-binding sites in prefrontal cortex and hippocampus.

Using [(123)I]IQNB SPECT, one group found decreased muscarinic receptor availability in unmedicated patients with schizophrenia as compared to controls in a variety of cortical and subcortical brain regions (Raedler *et al.*, 2003). Another group used GTP-γS binding to distinguish M2 and M3 muscarinic receptors and found no change in postmortem cortex from patients with schizophrenia as compared to controls, while finding a reduction in M1 (Scarr *et al.*, 2006). Other groups have found decreased pirenzapine binding in the hippocampus in brains of patients with schizophrenia (Crook *et al.*, 2000) and decreased M1 receptor mRNA in dorsolateral prefrontal cortex (Dean *et al.*, 2002) but not caudate nucleus (Dean *et al.*, 2000).

At the level of genetic findings, there is evidence for linkage of an M1 polymorphism to decreased performance on the Wisonsin Card Sort Test (Liao *et al.*, 2003). In addition, there is evidence that an M5 polymorphism confers susceptibility to schizophrenia. Interestingly, the M5 variant seems to confer risk only in combination with a nicotinic $\alpha 7$ polymorphism (De Luca *et al.*, 2004).

Finally, it has been reported that circulating antibodies to the M1 receptor can be identified in the serum of some schizophrenic patients. These antibodies

displace tritiated pirenzapine and have agonist-like properties at the M1 receptor in *in vitro* assays (Borda *et al.*, 2002).

B. Deficits in Components of Nicotinic Cholinergic Transmission

There is also ample evidence of alterations of nAChRs in the brains of patients with schizophrenia. The most notable set of findings pertains to decrements in α7-containing nAChRs and their function in sensory gating. These studies are dealt with in detail in the subsequent chapter by Martin and Freedman, this volume.

Apart from the well-defined case of α7-containing nicotinic receptors, more global changes in nicotinic cholinergic receptors have been observed as well. One group observed decreased high-affinity nicotine and epibatidine binding in postmortem brains from patients with schizophrenia as compared to controls (Breese *et al.*, 2000). The differences were seen in hippocampus, cortex, and caudate in the subgroup of patients versus controls who smoked. The same group reported increases in receptor sites in nicotine and haldol treated rats (Breese *et al.*, 2000). However, another group found elevation of nicotine binding in the striatum of patients with schizophrenia (Court *et al.*, 2000) and minimal changes in α-bungarotoxin binding in the thalamus (Court *et al.*, 1999).

C. Deficits in Cholinergic Innervation

Besides decrements in receptors for ACh, one group has observed a reduction in numbers of cholinergic interneurons in the ventral striatum (Holt *et al.*, 1999, 2005), but not in other striatal regions. However, cortical cholinesterase and ChAT activity is not reduced in the brains of patients with schizophrenia (Haroutunian *et al.*, 1994).

D. Summary

There are numerous findings of abnormalities in the expression or distribution of many components of cholinergic systems in the brains of patients with schizophrenia, the bulk of which would be expected to lead to decrements in cholinergic neurotransmission. It is unclear whether all of these are primary deficits or in some cases downstream effects of other lesions. At least some cholinergic deficits may have to interact with deficits in other systems in order to confer disease vulnerability. However, despite these uncertainties, the preponderance of evidence points toward possible roles for abnormalities in cholinergic systems participating in the pathophysiology of schizophrenia.

VI. Evidence for Cholinergic Contributions to Schizophrenia Pathophysiology from Clinical and Preclinical Psychopharmacology

Correlation of the efficacy of medications used to treat target symptoms of schizophrenia with effects of those medications on cholinergic systems has been another way in which investigators have attempted to infer potential roles for cholinergic systems in the pathophysiology of schizophrenia. The complex receptor-binding properties of antipsychotic medications and complex relationships of target symptoms and medication side effects have made it difficult to make such inferences in a clear and convincing way. That said, there seems to be ample evidence that well-chosen cholinergic targets have potential to be therapeutic targets.

A. ACh Release, Muscarinic Blockade, Partial Agonists, and "Atypicality"

Preclinical studies have demonstrated that many antipsychotic medications cause the release of ACh in cortical (Ichikawa *et al.*, 2002; Li *et al.*, 2005) and hippocampal regions (Johnson *et al.*, 2005; Shirazi-Southall *et al.*, 2002). While a broad range of antipsychotic medications have been shown to release cortical and hippocampal ACh, olanzapine and clozapine have been shown to be especially potent in this regard. Interestingly, these are the two antipsychotic medications which seem to have greater efficacy against a broader range of symptoms than other antipsychotic medications (Kane *et al.*, 1988, 2001; Lieberman *et al.*, 2003, 2005).

To some extent, antipsychotic-induced release of cortical ACh correlates with M2 binding affinity; antipsychotic medications with less M2 binding tend to be less potent inducers of cortical ACh release (Johnson *et al.*, 2005). Observations such as these combined with the observation that both clozapine and olanzapine bind muscarinic receptors with high affinity have led to the idea that anticholinergic properties of antipsychotic medications might be responsible for ACh release, and further might be correlated with "atypicality." This scenario is plausible in that muscarinic receptors can serve as inhibitory presynaptic autoreceptors, providing a potential mechanism by which their blockade could augment ACh release.

However, olanzapine and clozapine may be only weakly antimuscarinic at the level of clinical symptoms, with fewer anticholinergic side effects than expected based on their potent *in vitro* displacement of muscarinic ligands (Bymaster *et al.*, 1996). This discrepancy is likely due both to subtype selectivity at muscarinic sites, and to partial agonist activity at M4 and M2 receptors (Bymaster *et al.*, 2003; Michal *et al.*, 1999).

Interestingly, clozapine has cognition impairing properties in mice, but the effect is complex, in that clozapine reduces scopolamine-induced impairment while its direct effects on cognition are reversed by cholinesterase inhibitors. This pattern of reduction of antagonist effects and reversal by treatments that increase the endogenous ligand is highly suggestive of partial agonist effects (Ninan and Kulkarni, 1996) or of interaction of ACh with other types of muscarinic and/or nicotinic receptors (see above). Of note, at least two other compounds, xanomeline (reviewed in Mirza *et al.*, 2003) and PTAC (Bymaster *et al.*, 1999), show antipsychotic-like activity in animal models and are also partial agonists at M4 and M2 receptors.

A further wrinkle in this data is introduced by the fact that both clozapine and olanzapine still release cortical and hippocampal ACh in mice lacking M2 and M4 receptors (Bymaster *et al.*, 2003). As such, neither blockade nor partial agonism at these sites appears to be required for ACh release. In view of recent studies implicating both presynaptic nicotinic and dopamine receptors in the modulation of ACh release, the potential contribution of these pathways to the effects of clozapine and olanzapine should, perhaps, be considered.

B. Both Procholinergic and Anticholinergic Compounds May Ameliorate or Worsen Different Symptom Domains

Adding further to confusion about the role of cholinergic transmission in schizophrenia, muscarinic agonists and antagonists exert opposing effects on various schizophrenia symptom clusters. There has been extensive interest in this area because of the use of medications with "anticholinergic" properties as antipsychotics (e.g., chlorpromazine, perphenazine) and because of the use of medications such as benztropine and biperiden to counter the parkinsonian side effects of traditional neuroleptic antipsychotics. For some time, the prevailing opinion seems to have been that anticholinergic effects were fairly neutral in relation to symptoms of schizophrenia, but closer examination has revealed a more complicated picture.

Several studies showed some increase in positive symptoms (hallucinations and delusions) when anticholinergic medications were added to neuroleptics. In a placebo-controlled trial, procyclidine or placebo was added to flupenthixol in a group of 36 patients, with the subsequent finding that those receiving procyclidine had more positive symptoms than those receiving placebo (Johnstone *et al.*, 1983). Another study compared symptoms in 47 patients receiving neuroleptics during periods with and without treatment with benztropine or trihexyphenidyl. Again, the anticholinergic medication was associated with an increase in positive symptoms (Singh *et al.*, 1987). A study showed that biperiden increased positive symptoms in a small group of schizophrenic patients when added during a medication free

period, strengthening the case that the symptomatic worsening is a direct anticholinergic effect rather than one that depends on interaction with the effects of other medications (Tandon *et al.*, 1991).

Anticholinergic compounds such as benztropine have been shown to cause memory impairment (Brebion *et al.*, 2004; Tune *et al.*, 1982). In one small study, higher serum anticholinergic levels correlated with worsened recall but improved reaction time (Strauss *et al.*, 1990). It is possible that improvements in reaction time represent an anti-parkinsonian effect as all patients in the study were taking antipsychotic medications. In another study, single injections of benztropine or glycopyrrolate impaired free recall (McEvoy and Freter, 1989). Overall, it appears likely that anticholinergic medications could worsen some cognitive deficits in schizophrenia.

At the same time, there is a small body of evidence showing that anticholinergic may decrease some negative symptoms in schizophrenia. This provides an instance in which the domain of cognitive symptoms and the domain of negative symptoms can be separated from one another in part based on how they are affected by pharmacological treatment.

In one study, treatment of a small number of patients with trihexyphenidyl resulted in decreases in affective flattening, avolition, and anhedonia/asociality (Tandon *et al.*, 1992). In another study by the same group, biperiden also reduced negative symptoms (while increasing positive symptoms; Tandon *et al.*, 1991). Abuse of trihexyphenidyl and benztropine has at times been cited as "self-medication" of negative symptoms by patients with schizophrenia, and indeed in one study those who abused these medications tended to have higher Brief Psychiatric Rating Scale (BPRS) scores and more negative symptoms than those who did not abuse anticholinergic medications (Zemishlany *et al.*, 1996).

One might speculate that actions of anticholinergic medications against negative symptoms reflect activity in psychomotor circuits that are parallel in some way to the motor circuits in which anticholinergic medications oppose the parkinsonian actions of neuroleptics.

Conversely, muscarinic *agonists* have been proposed to have antipsychotic activity and may have potential effects against positive symptoms with particular attention paid to the compound xanomeline (reviewed in Bymaster *et al.*, 2002, 2003).

Recent findings on the function of muscarinic receptors in the striatum may shed some light on these apparent paradoxes. The recent paper by Wang and coworkers (also discussed in Section III.B of this chapter) shows that D2 dopamine receptors serve to inactivate striatal cholinergic interneurons which signal to M1 muscarinic receptors on MSpNs. In essence, muscarinic stimulation and blockade of dopamine are functional equivalents in this circuit. The muscarinic receptors reduce intracellular calcium in the MSpNs, which in turn reduces the production of endocannabinoids, reducing depolarization induced suppression

and long-term depression of neurotransmission. In summary, cholinergic activation in this circuit results in more activity of MSpNs in the indirect pathway—and subsequently less release of inhibition of the corticostriatal pathways that may convert drives and feelings into actions and perceptions. One might predict that this action would reduce positive symptoms in schizophrenia in a manner analogous to that of D2 blockade by neuroleptics, but one might also predict that in some components of these pathways, the same set of actions could result in an increase in negative symptoms (Wang *et al.*, 2006).

While far from clear, the body of evidence on effects of muscarinic agonists and antagonists in schizophrenia, and on the cholinergic binding properties of antipsychotic medications seems to point to a complex role for cholinergic transmission in different domains of psychopathology with loci of action in the brain likely to depend on the specific symptom domain examined. A challenge to neuropyschopharmacologists will be to find ways to balance and dissociate beneficial and harmful effects of blocking and enhancing cholinergic transmission at muscarinic receptors.

C. Nicotine Ameliorates a Wide Range of Deficits Seen in Schizophrenia

A large part of the pharmacological evidence pointing to potential cholinergic roles in schizophrenia pathophysiology concerns salutary effects of nicotine in patients with schizophrenia. Nicotine affects a wide range of symptom domains and neuropsychological findings. We will give a broad sampling of the data here, and refer the reader to the subsequent chapter by Martin and Freedman, this volume, for an in-depth review of data on nicotinic receptors and the processing of sensory information in schizophrenia.

Nicotine has been shown to improve abnormalities in smooth pursuit eye movement and saccades during visual tracking (Avila *et al.*, 2003; Depatie *et al.*, 2002; Larrison-Faucher *et al.*, 2004; Sherr *et al.*, 2002). The improvement in saccades was independent of the smoking status of the patients, thus addressing the possibility that nicotine's effect resulted directly from the elevated incidence of smoking by people with schizophrenia. Nicotine also improved sustained attention in these visual tasks (Avila *et al.*, 2003; Depatie *et al.*, 2002). The effects of nicotine on performance of visual tracking and tasks of visual attention may involve hippocampus and cingulate gyrus (Levin *et al.*, 2006; Newhouse *et al.*, 2004; Tanabe *et al.*, 2006).

In another domain, nicotine improved performance of tasks involving working memory in schizophrenic subjects, enhancing task-related activation of thalamus and anterior cingulated cortex as seen on fMRI (Jacobsen *et al.*, 2004).

Nicotine has also been shown to reverse haloperidol-induced impairments in reaction time and working memory (Levin *et al.*, 1996). Nicotine nasal

spray improved delayed recognition and spatial working memory in schizophrenic patients (Myers *et al.*, 2004; Smith *et al.*, 2006). Interestingly, nicotine may actually impair working memory in otherwise healthy smokers (Park *et al.*, 2000) suggesting an inherent difference in nicotine responses in the brains of persons with schizophrenia (discussed in Mansvelder *et al.*, 2006; Newhouse *et al.*, 2004).

While numerous studies show beneficial effects of administered nicotine in schizophrenia, the reverse is not true, that is to say, nicotine withdrawal did not increase positive symptoms in a group of patients with schizophrenia who quit smoking. Increases in negative symptoms were modest and transient (Dalack *et al.*, 1999). In considering these results, it may be important to take into account that the frequent high peaks of nicotine delivered by smoking may not be of sustained therapeutic benefit as compared to other systems of delivery.

Finally, the fairly extensive data on sensory processing and nicotine is perhaps the best pharmacological evidence for a role of cholinergic systems in schizophrenia. It is made stronger by the linkage of a polymorphism in the $\alpha 7$ nicotinic receptor subunit gene to sensory gating deficits in patients with schizophrenia and their relatives (Freedman *et al.*, 2003; see chapter by Martin and Freedman, this volume). This topic is reviewed in detail in the subsequent chapter by Martin and Freedman, this volume. Overall, and in contrast to the data on muscarinic AChRs, evidence to date strongly supports the notion that treatments that interact with nAChRs have almost uniformly ameliorative effects on symptoms of schizophrenia.

D. Despite Clear Effects of Other Cholinergic Compounds, Cholinesterase Inhibitors Are Not Proven Adjuncts in the Treatment of Schizophrenia

Further pharmacological evidence concerning cholinergic participation in the pathophysiology of schizophrenia comes from studies in which patients were treated with medications from the family of AChE inhibitors which were initially developed to treat Alzheimer's disease (Coyle and Kershaw, 2001; Crismon, 1994; Dooley and Lamb, 2000; Jann, 2000). The effects of these medications in patients with schizophrenia are equivocal at best.

There are several case reports describing improvement in negative and cognitive symptoms of individual patients with cholinesterase inhibitors (Rosse and Deutsch, 2002, using galantamine). There are some positive open label trials of rivastigmine (Lenzi *et al.*, 2003; Mendelsohn *et al.*, 2004) in which patients show improvements on standard-rating scales such as Positive and Negative Symptom

Scale (PAANS) or BPRS. The study by Lenzi *et al.* (2003) was focused on quality of life measures. The study by Mendelsohn *et al.* (2004) was limited to patients with comorbid dementia, so it may be difficult to generalize the result to the schizophrenic population as a whole.

Some positive findings involving cholinesterase inhibitors were in studies that focused on a specific neurocognitive endophenotype rather than on clinical outcome as a whole. One group found that donepezil normalized fMRI findings on a verbal fluency task (Nahas *et al.*, 2003) and another group found that rivastigmine improved performance on a sustained attention task (Aasen *et al.*, 2005).

On the negative side, several reports of placebo-controlled, double-blind crossover trials of donepezil show no efficacy against symptoms of schizophrenia. These studies were all done using donepezil as a neuroleptic augmentation treatment. Some (Mazeh *et al.*, 2006; Stryjer *et al.*, 2003) were in elderly patients or patients with known comorbid dementia. Others were in a general population of stable schizophrenic patients (Friedman *et al.*, 2002; Stryjer *et al.*, 2004; Tugal *et al.*, 2004). No effects were seen on positive, negative, or cognitive symptoms. All of the studies were fairly small, and in some cases there is concern about potential confounding effects of concurrent nicotine use by patients.

Overall, at this time there is little evidence to suggest significant benefits of cholinesterase inhibitors in schizophrenia, especially to patients who do not suffer from comorbid dementia. In some respects, given what we have outlined about the complexity of cholinergic systems, it is not surprising that a class of medications which brings about global increases in ACh levels would have modest effects; in many brain locations, presynaptic inhibition of release may compensate for decreased degradation when ACh levels rise. Thus, given the evidence of effects of treatments targeting nicotinic and muscarinic receptors, it would seem unwarranted to view the modest effects of cholinesterase inhibitors as evidence against participation of cholinergic systems in schizophrenia pathophysiology.

VII. Conclusions

Proper function of cholinergic systems in the brain is essential for a variety of neurocognitive tasks that are impaired in schizophrenia including attention, volition, working memory, assignment of salience, and the processing of sensory information.

While it is unlikely that cholinergic deficits alone account for any particular symptom domain in schizophrenia, there is ample evidence that schizophrenia is

associated with genetic changes and brain abnormalities that can influence both the development and function of cholinergic systems, and that interaction of cholinergic deficits with deficits in other systems has the potential to produce disease symptoms.

Perhaps the clearest case of a cholinergic deficit is that of abnormality in the control of α7 nicotinic receptor expression conferring deficits in sensory gating and vulnerability to schizophrenia (cf chapter by Martin and Freedman, this volume). However, it is likely that nicotine has other important sites of action relating to schizophrenia, and that muscarinic effects on corticostriatal and other circuits are of independent import.

Inferences made from clinical and preclinical psychopharmacological data about the performance of cholinergic systems in schizophrenia are fraught with difficulty and do not point to a simple dysregulation of cholinergic transmission at a single brain location. Rather, there are numerous points where dysfunction of particular components of cholinergic signaling can contribute to symptoms or where medications can ameliorate (or worsen) symptoms regardless of an intrinsic cholinergic deficit. In some instances, these points are uncomfortably close to one another and the effects of cholinergic signaling may be arrayed in opposite directions. In other instances, manipulation of cholinergic systems at one site may be undone by effects of the same manipulation at a distant site. The challenge for neurobiologists and pyschopharmacologists is to find ways to refine our interventions in this complex system and to develop compounds or combinations of compounds which can target sites of interest, without substituting one set of impairments for another.

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SCHIZOPHRENIA AND THE $\alpha 7$ NICOTINIC ACETYLCHOLINE RECEPTOR

Laura F. Martin*,† and Robert Freedman*,†

*Research Service, VA Eastern Colorado Health Care System
Denver, Colorado 80220, USA
†Department of Psychiatry, University of Colorado at Denver Health Sciences Center
Denver, Colorado 80262, USA

In addition to the devastating symptoms of psychosis, many people with schizophrenia also suffer from cognitive impairment. These cognitive symptoms lead to marked dysfunction and can impact employability, treatment adherence, and social skills. Deficits in P50 auditory gating are associated with attentional impairment and may contribute to cognitive symptoms and perceptual disturbances. This nicotinic cholinergic-mediated inhibitory process represents a potential new target for therapeutic intervention in schizophrenia. This chapter will review evidence implicating the nicotinic cholinergic, and specifically, the $\alpha 7$ nicotinic receptor system in the pathology of schizophrenia. Impaired auditory sensory gating has been linked to the $\alpha 7$ nicotinic receptor gene on the chromosome 15q14 locus. A majority of persons with schizophrenia are heavy smokers. Although nicotine can acutely reverse diminished auditory sensory gating in people with schizophrenia, this effect is lost on a chronic basis due to receptor desensitization. The $\alpha 7$ nicotinic agonist 3-(2,4 dimethoxy)benzylidene-anabaseine (DMXBA) can also enhance auditory sensory gating in animal models. DMXBA is well tolerated in humans and a new study in persons with schizophrenia has found that DMXBA enhances both P50 auditory gating and cognition. $\alpha 7$ Nicotinic acetylcholine receptor agonists appear to be viable candidates for the treatment of cognitive disturbances in schizophrenia.

INTERNATIONAL REVIEW OF NEUROBIOLOGY, VOL. 78
DOI: 10.1016/S0074-7742(06)78008-4

0074-7742/07 $35.00

I. Introduction

In addition to the more obvious symptoms of hallucinations and delusions, people with schizophrenia frequently suffer from cognitive symptoms such as the inability to focus attention. This results in a "flooding" with extraneous sensory stimuli which overwhelms the person's ability to think coherently (Venables, 1992). Poor cognitive functioning contributes to both poor role-functioning and high costs of care through its association with activities of daily living, productivity, rate of inpatient hospitalization and outpatient utilization, independence, trainability/education levels, employability, and the lost productivity of family members spent caring for their ill relatives (reviewed in Sevy and Davidson, 1995). Cognitive impairment also contributes to poor medication adherence (Jeste *et al.*, 2003) and limits the efficacy of rehabilitative therapies (reviewed in Sharma and Antonova, 2003). Cognitive deficits improve slightly with current antipsychotic medications, but they are not normalized and therefore remain a target for new treatment efforts (Weickert *et al.*, 2003). Given increasing evidence for a role of the nicotinic cholinergic system's role in the cognitive symptoms of schizophrenia, the $\alpha 7$ nicotinic acetylcholine receptor has been proposed as a candidate for the development of medications specifically targeting cognitive deficits in schizophrenia (Martin *et al.*, 2004). This chapter will review the neurobiological findings that led to the development of this promising new drug treatment for schizophrenia as well as new evidence for the beneficial effect of an $\alpha 7$ nicotinic receptor agonist on cognitive impairment in schizophrenia.

II. Neurobiological and Neurogenetic Evidence for a Link Between the $\alpha 7$ Nicotinic Acetylcholine Receptor and Schizophrenia

Sensory gating, measured using the P50 auditory-evoked response, is impaired in persons with schizophrenia (Adler *et al.*, 1985). The P50 auditory-evoked response occurs 40–75 ms following an auditory stimulus. When a second auditory stimulus is presented in close proximity (500 ms), the P50 auditory-evoked response to the second stimulus is diminished, which is evidence for the activity of an inhibitory process. This impairment has been replicated in multiple independent laboratories (Boutros *et al.*, 1991; Clementz *et al.*, 1997; Judd *et al.*, 1992; Louchart-de la Chapelle *et al.*, 2005; Ward *et al.*, 1996) and is present by the first episode of psychosis (Yee *et al.*, 1998). This inhibitory failure is associated with poor sustained attention, as measured by diminished performance on the Digit Vigilance Test and other tests of attentional dysfunction (Cullum *et al.*, 1993; Yee *et al.*, 1998).

Evidence for the role of the α7 nicotinic acetylcholine receptor in auditory gating was initially established using multiple animal models. The auditory-evoked response of hippocampal CA3 pyramidal neurons in the rat, the P20-N40 field potential, parallels the properties of the human P50 auditory-evoked response. The α7 nicotinic receptor antagonist α-bungarotoxin disrupts P20-N40 gating, while the nicotinic receptor channel blocker mecamylamine and the muscarinic antagonist scopolamine have no effect on P20-N40 gating (Luntz-Leybman *et al.*, 1992). The DBA/2 strain of mice has genetically decreased levels of α7 nicotinic receptors in the CA3 region and impaired auditory gating (Stevens *et al.*, 1996). Finally, nicotine restores auditory gating in fimbria-fornix lesioned rats with impaired auditory gating due to the loss of cholinergic innervation to the hippocampus (Bickford and Wear, 1995).

α7 Nicotinic receptors mediate this inhibitory processing by enhancing the release of gama-aminobutyric acid (GABA) from GABAergic interneurons via a postsynaptic, calcium-dependent mechanism (Albuquerque *et al.*, 1998; Frazier *et al.*, 1998). Nitric oxide prolongs this effect through a second messenger system (Adams *et al.*, 2000). This enhanced release of GABA stimulates $GABA_B$ receptors which in turn decreases the release of glutamate (Hershman *et al.*, 1995). This effect is thought to prevent hippocampal neurons from responding to the second stimulus in the auditory gating paradigm. These nicotinic receptor-mediated interactions between inhibitory (GABA) and excitatory (glutamate) neurons are also proposed to play a role in the efficiency and patterning of neuronal functioning within the hippocampus and cortex (Albuquerque *et al.*, 2000; Alkondon *et al.*, 2000; Ji and Dani, 2000; Jones *et al.*, 1999).

A parallel series of studies in humans also implicated the α7 nicotinic acetylcholine receptor in the physiology of P50 auditory gating. Nicotine gum and physostigmine were found to improve gating in the relatives of persons with schizophrenia who also had impaired auditory gating (Adler *et al.*, 1992). The study of this group of relatives was especially useful as it was able to avoid the confounds of the additional pathological effects of schizophrenia, the effects of chronic neuroleptic treatment as well as the effects of chronic smoking on nicotinic receptor levels. These findings were extended to persons with schizophrenia (Adler *et al.*, 1993). Next, mecamylamine was administered with nicotine at a dose which blocks α4/β2 receptors. Mecamylamine did not attenuate the nicotine induced enhancement of auditory gating. Therefore, the α7 nicotinic receptor appears to be the primary cholinergic receptor responsible for P50 auditory gating in humans as well (Freedman *et al.*, 1994).

In addition to the α7-mediated deficits in P50 auditory gating, people with schizophrenia also have abnormalities in the expression of central nervous system nicotinic receptors. Decreased α7 nicotinic receptor binding has been noted in the reticular nucleus of the thalamus (Court *et al.*, 1999), the hippocampus (Freedman *et al.*, 1995), and the cingulate cortex (Marutle *et al.*, 2001).

Reduced $\alpha 7$ subunit levels have been noted in frontal lobe regions (Guan *et al.*, 1999), including the dorsolateral prefrontal cortex (Martin-Ruiz *et al.*, 2003). Reduced levels of mRNA are also seen in peripheral blood lymphocytes (Perl *et al.*, 2003).

The relatives of persons with schizophrenia also have poor P50 auditory gating (Clementz *et al.*, 1998; Ross *et al.*, 1999; Siegal *et al.*, 1984), consistent with a genetically determined trait (Waldo *et al.*, 1991). An initial genome scan using poor P50 auditory gating as a phenotype gave only suggestive results at several chromosomes (Coon *et al.*, 1993). Following the identification of genetic markers specific to the $\alpha 7$ nicotinic acetylcholine receptor gene (CHRNA-7) at 15q13–14 (Chini *et al.*, 1994), P50 auditory gating was linked to the chromosome 15q14 locus of CHRNA-7 (Freedman *et al.*, 1997). Families from the NIMH Schizophrenia Genetics Initiative database have since been utilized to find linkage to the diagnosis of schizophrenia itself (Leonard *et al.*, 1998). Since that time, replications of these findings have occurred in North American families of African descent (Kaufmann *et al.*, 1998) and European descent (Tsuang *et al.*, 2001), German families (Stöber *et al.*, 2000), South African families (Riley *et al.*, 2000), Azorean families (Xu *et al.*, 2001), Taiwanese families (Liu *et al.*, 2001) and Canadian families (De Luca *et al.*, 2004). Other studies, including those that have looked specifically at this region, have not found linkage (Curtis *et al.*, 1999; Neves-Pereira *et al.*, 1998).

Although no amino acid-coding region polymorphisms have been identified, multiple single nucleotide polymorphisms in the promoter region of CHRNA-7 as well as a partial duplication of the CHRNA-7 gene have been characterized (Gault *et al.*, 1998). Certain alleles are more frequently present in people with schizophrenia and their family members (Houy *et al.*, 2004; Leonard *et al.*, 2002). Furthermore, as some of these alleles are associated with both decreased promoter region activity *in vitro* and impaired P50 auditory gating, they represent functional polymorphisms that may be related to brain inhibitory pathway failure (Leonard *et al.*, 2002).

III. The Prototypic $\alpha 7$ Nicotinic Agonist, Nicotine, and Schizophrenia

The frequency of tobacco smoking is elevated in people with schizophrenia in both inpatient (De Leon *et al.*, 1995; Llerena *et al.*, 2003) and outpatient settings (Diwan *et al.*, 1998; Hughes *et al.*, 1986). They are heavier smokers (De Leon *et al.*, 1995; Kelly and McCreadie, 1999; Lasser *et al.*, 2000; Masterson and O'Shea, 1984) and they extract more nicotine per cigarette smoked than the general population (Olincy *et al.*, 1997; Strand and Nybäck, 2005 but see Bozikas *et al.*, 2005). In addition to the health implications of smoking (Goff *et al.*, 2005), the burden of this heavy use includes spending 27% of an already limited income on

the purchase of cigarettes (Steinberg *et al.*, 2005). Their motivation to quit smoking is low (Addington *et al.*, 1997; Ziedonis and George, 1997), and the smoking cessation rate is lower than the rates of other mentally ill populations (Diwan *et al.*, 1998) and the general population (Kelly and McCreadie, 1999). Fortunately, interventions targeted specifically for persons with schizophrenia are being developed (Steinberg *et al.*, 2004; Ziedonis *et al.*, 2003). Successful interventions have utilized cognitive behavioral therapy and sustained release bupropion (Evins *et al.*, 2001; Weiner *et al.*, 2001), nicotine replacement therapy (Breckenridge, 1990; Chou *et al.*, 2004; George *et al.*, 2000; Williams *et al.*, 2004; Ziedonis and George, 1997), cognitive behavioral therapy alone (Addington *et al.*, 1998), and contingent monetary reinforcement (Tidey *et al.*, 2002) to reduce smoking or promote abstinence. The reduction in smoking achieved may last for up to 2 years following the cessation treatment and is associated with a greater likelihood of abstaining in the future (Evins *et al.*, 2004).

The high rate and heavy level of smoking seen in this population may be related to the illness or its treatment (reviewed in Dalack *et al.*, 1998). Patients report that they smoke as a sedative, to reduce negative symptoms, and to counteract medication side effects (Forchuk *et al.*, 2002). Some investigators have hypothesized that smoking in people with schizophrenia may be their striving to reduce neuroleptic-induced side effects such as iatrogenic parkinsonism (Decina *et al.*, 1990; Goff *et al.*, 1992). Others have hypothesized that smoking may be an attempt to prevent worsening of their symptoms during nicotine withdrawal (Dalack *et al.*, 1999; Dalack and Meador-Woodruff, 1996) or an endeavor to alleviate symptoms of depression, anxiety, anhedonia, or amotivation (Glassman, 1993; Nisell *et al.*, 1995; Svensson *et al.*, 1990; Tung *et al.*, 1990). Finally, smoking may be a strategy to improve cognition (Nomikos *et al.*, 2000; Taiminen *et al.*, 1998) and sensory gating (Adler *et al.*, 1993).

Systematic studies of these hypotheses involving the administration (or withdrawal) of nicotine have demonstrated positive effects on movement disorders, negative symptoms, some cognitive tasks, sensory gating, and eye movement performance. Nicotine patch administration can improve tremor, bradykinesia-rigidity, and akathesia (Anfang and Pope, 1997; Yang *et al.*, 2002). However, one study found a worsening of Abnormal Involuntary Movement Scale scores following nicotine patch administration (Dalack *et al.*, 1999) and another study found no effect of smoking on tardive dyskinesia, extrapyramidal or parkinsonian symptoms (Smith *et al.*, 2002). Nicotine withdrawal as part of an abstinence or harm reduction treatment may exacerbate psychotic and depressive symptoms (Evins *et al.*, 2001), although this exacerbation may be prevented by the use of nicotine replacement (George *et al.*, 2000) or bupropion (Evins *et al.*, 2001; Weiner *et al.*, 2001). The use of a nicotine patch in a research setting does not affect Brief Psychiatric Rating Scale scores or Scale for the Assessment of Negative Symptoms SANS; (Dalack *et al.*, 1999; Yang *et al.*, 2002). A case report

found that adjuvant galantamine, the anticholinesterase inhibitor and allosteric nicotinic receptor modulator, improved the SANS score (Rosse and Deutsch, 2002).

The effects of nicotine on neuropsychological measures in persons with schizophrenia have been mixed. Abstinence and then reinitiation of smoking had no effect on attentional measures (Sacco *et al.*, 2005), the nicotine patch improved attention (Dépatie *et al.*, 2002; Levin *et al.*, 1996), nicotine gum worsened attention in smokers and improved it in nonsmokers (Harris *et al.*, 2004), and nicotine nasal spray had no effect on attention (Sherr *et al.*, 2002). Smoking abstinence impaired working memory (George *et al.*, 2001; Sacco *et al.*, 2005) and the reinstatement of smoking improved performance (Sacco *et al.*, 2005). The nicotine patch also improved haloperidol-induced deficits on another test of working memory (Levin *et al.*, 1996). A functional magnetic resonance imaging study of an auditory working memory task found a behavioral improvement following nicotine patch that was associated with increased activation within the insula, putamen, and thalamus (Jacobsen *et al.*, 2004). Nicotine nasal spray, however, had no effect on working memory (Myers *et al.*, 2004). While one study found a positive effect for nicotine nasal spray on verbal memory (Smith *et al.*, 2002), this effect was not seen for smoking (Sacco *et al.*, 2005; Smith *et al.*, 2002), nicotine gum (Harris *et al.*, 2004), or the nicotine patch (Levin *et al.*, 1996). Both nicotine nasal spray and the patch appear to improve complex reaction times (Levin *et al.*, 1996; Smith *et al.*, 2002), but there is no effect on simple reaction time (Levin *et al.*, 1996). Neither abstinence (George *et al.*, 2001) nor the reinitiation of smoking affects executive functioning (Sacco *et al.*, 2005). Finally, one study found an improvement in a visuospatial delayed recognition task following nicotine nasal spray in smokers (Myers *et al.*, 2004) while another study found no effect of nicotine gum on visuospatial abilities (Harris *et al.*, 2004).

Studies of the effect of nicotine on physiological abnormalities such as sensory gating and eye tracking have been more consistent. One of the first investigations of the effect of nicotine in persons with schizophrenia found that abnormal P50 auditory gating was normalized in persons with schizophrenia after smoking (Adler *et al.*, 1993). This finding was replicated using the nicotine patch (Griffith *et al.*, 1998). In a different paradigm of sensory gating, prepulse inhibition, smoking prior to testing results in better test performance than not smoking (Kumari *et al.*, 2001). Studies of smooth pursuit eye movements have been equally robust, with every study to date finding significant enhancement of performance using cigarette smoking (Olincy *et al.*, 1998, 2003), nicotine nasal spray (Avila *et al.*, 2003; Sherr *et al.*, 2002), and nicotine patch (Dépatie *et al.*, 2002). A functional magnetic resonance imaging study of the effects of nicotine on smooth pursuit eye movements found that nicotine enhanced cingulate and precuneus activation and decreased abnormally elevated hippocampal activation

(Tregellas *et al.*, 2004, 2005). Antisaccade task performance also improved following the administration of nicotine gum (Larrison-Faucher *et al.*, 2004).

While the physiological studies are all positive, the neurocognitive findings are less consistent. These studies are limited by the difficulties inherent in studies of any pharmacological agent, such as dosing and administrative route, as well as the specific difficulty present in administering nicotine in persons who are already dependent on the substance. One way to control for these difficulties is to use a population that is not dependent on nicotine such as nonsmokers with schizophrenia. While diminishing the generalizability of the results as well as making recruitment more difficult, this avoids the confounds of withdrawal and different long-term biological effects of smoking such as receptor upregulation and desensitization. Adler *et al.* (1992) took this approach one step further by first examining the effects of nicotine gum in the first-degree relatives of persons with schizophrenia who were impaired on the P50 auditory gating paradigm, thereby avoiding the additional confounds of the illness itself and the medications used to treat the illness. If one chooses to use schizophrenics who smoke, two types of difficulties arise. The first is how to deal with the issue of withdrawal. One must find a balance between clearing the system of the acute effects of nicotine while not precipitating symptoms of withdrawal that might affect performance. Our laboratory has advocated the use of a 2-h period of abstinence to balance these demands. The second issue is how to control for smoking status if using a comparison group of control smokers. While the test-retest reliability of the reported smoking history in persons with schizophrenia is quite high (0.92–0.99) and the intercorrelation of objective measures of smoking heaviness such as carbon monoxide, urine cotinine, and nicotine are fairly similar between persons with schizophrenia and controls (0.52–0.80), the relationship between the reported number of cigarettes smoked per day and these objective measures is much lower for persons with schizophrenia (0.02–0.37 vs 0.61–0.65; Yang *et al.*, 2003). Although not studied directly, this may be due to the greater extraction of nicotine in persons with schizophrenia (Olincy *et al.*, 1997). Despite these difficulties, however, there appears to be clear normalization of deficits in persons with schizophrenia following cigarette smoking or the administration of nicotine.

Nicotine, however, has several limitations as a therapeutic agent. Nicotine induces tachyphylaxis, as demonstrated by the inability of repeated dosing of nicotine to enhance impaired P50 auditory gating. Therefore, sustained benefit does not occur. While nicotine replacement eliminates many of the risks of the other ingredients and additives in tobacco, the long-term risks of chronic nicotine use are unknown and may include carcinogenic risk (Crowley-Weber *et al.*, 2003; Heusch and Maneckjee, 1998) and cerebro- or cardiovascular risk (Benowitz, 2003; Benowitz and Gourlay, 1997; Chalon *et al.*, 2000; Elliott *et al.*, 2003;

Fang *et al.*, 2003; Hakki *et al.*, 2002; West *et al.*, 2003). Furthermore, nicotine is an addictive agent, and the development of tolerance can lead to the stressful symptoms of withdrawal in the absence of continued nicotine dosing (Benowitz, 1998). Less potentially toxic and more chronically effective cholinergic treatments are needed.

An alternative to the use of nicotine as a nicotinic agonist would be to increase endogenous release of acetylcholine. For instance, clozapine is able to increase acetylcholine levels in hippocampus (Shirazi-Southall *et al.*, 2002). Consistent with this acetylcholine-enhancing effect, patients on clozapine have near normal levels of P50 suppression. The normalization of auditory gating over time parallels clinical improvement (Nagamoto *et al.*, 1999). Typical neuroleptics and the majority of atypical neuroleptics, however, have no effect on P50 auditory gating (Adler *et al.*, 2004; Freedman *et al.*, 1983; Yee *et al.*, 1998). Clozapine-induced normalization of auditory gating in DBA/2 mice is blocked by α-bungarotoxin, implicating an $\alpha 7$ nicotinic receptor mechanism (Simosky *et al.*, 2003). Ondansetron, an antiemetic, also increases acetylcholine levels via 5HT-3 receptor antagonism. Similar to clozapine, it enhances P50 auditory gating in persons with schizophrenia (Adler *et al.*, 2005). The anticholinesterase inhibitor donepezil nonsignificantly enhanced the P50 auditory gating in persons with schizophrenia (Buchanan *et al.*, 2002). Although olanzapine is also able to increase acetylcholine levels in the hippocampus (Shirazi-Southall *et al.*, 2002), and a cross-sectional study has shown less impaired levels of auditory gating in people with schizophrenia treated with olanzapine (Light *et al.*, 2000), a more definitive cause and effect relationship has not been demonstrated with a longitudinal study (Arango *et al.*, 2003) and a second cross-sectional study found no differences between unmedicated schizophrenics and patients taking olanzapine (Adler *et al.*, 2004).

Interestingly, clozapine has also shown efficacy in its ability to reduce smoking levels in some (Combs and Advokat, 2000; George *et al.*, 1995; McEvoy *et al.*, 1999) but not every study of persons with schizophrenia (De Leon *et al.*, 2005). An additional study examining the effects of bupropion on smoking rates in persons with schizophrenia was confounded by clozapine use. The one abstinent person at 3 months and three of the four abstinent persons at a 2 year follow-up were also taking clozapine (Evins *et al.*, 2001, 2004). These findings may be consistent with a decreased need to self-medicate with cigarette smoking. However, this effect may not be unique to clozapine, as olanzapine and risperidone have also been shown to be associated with greater abstinence when compared to typical antipsychotics (George *et al.*, 2000). Despite its superior efficacy (Kane *et al.*, 1988) and these additional proposed benefits, treatment with clozapine is limited given the significant side effects of sedation, drooling, tachycardia, and weight gain as well as the serious potential side effects of seizures and agranulocytosis.

IV. The Search for an α7 Nicotinic Acetylcholine Receptor Agonist

Two compounds in current clinical use may have direct effects on α7 nicotinic receptors. The anticholinesterase inhibitor galantamine, which has additional modulatory effects on the α7 nicotinic receptor, has been reported to be beneficial for schizophrenia in a case study (Rosse and Deutsch, 2002). Tropisetron, a 5-HT_3 antagonist marketed outside the United States as an antinausea drug, also has efficacy as an α7 nicotinic receptor agonist (Macor *et al.*, 2001; Papke *et al.*, 2005). Tropisetron increases the inhibition of P50 auditory gating in schizophrenia (Koike *et al.*, 2005), an effect due to actions at the α7 nicotinic receptor (Hashimoto *et al.*, 2005).

In addition to these medications already being clinically utilized, several cholinergic receptor agonists have been developed to further characterize central nervous system cholinergic function and as potential candidates for the treatment of dementia of the Alzheimer's type (Kem, 2000). Drugs currently in development include a 1,4-diaza-bicyclo[3.2.2]nonane-4-carboxylic acid 4-pyridin-2-yl-phenyl ester at Pfizer Inc., an (E)-*N*-methyl-5 (3-pyridinyl)-4-penten-2-amine at Targacept Inc., and a substituted-heteroaryl-7-aza[2.2.1]bicycloheptanes at Pharmacia & Upjohn Company. AR-R 17779, an Astra Arcus product, is an acetylcholine analogue with full agonist properties at the α7 nicotinic receptor (Mullen *et al.*, 2000). ABT-418, while primarily functioning as an α4/β2 agonist, also has some agonist properties at the α7 nicotinic receptor (Briggs *et al.*, 1995). Derivatives of the 5-HT_3 receptor antagonist tropisetron are currently in development (Macor *et al.*, 2001). 3-(2,4 Dimethoxy)benzylidene-anabaseine (DMXBA) is one of a series of compounds derived from anabaseine, an alkaloid found in marine worms (Kem *et al.*, 1971, 1997; Meyer *et al.*, 1998c). DMXBA is a partial agonist at the α7 receptor (Briggs *et al.*, 1995; De Fiebre *et al.*, 1995) and is a weak competitive antagonist at α4/β2 nicotinic (Kem *et al.*, 1996; Meyer *et al.*, 1998a; Papke *et al.*, 2000) and 5HT-3 receptors. Although the metabolites of DMXBA are also active at these receptors, their biological effect may be limited by their greater polarity and consequently, greater difficulty in crossing the blood-brain barrier (Kem *et al.*, 2004).

The efficacy of α7 nicotinic receptor agonists has also been assessed in multiple animal paradigms of learning and memory (Levin and Rezvani, 2000; Levin and Simon, 1998). DMXBA improves memory-related behaviors in multiple paradigms, including a delayed matching to sample task (Briggs *et al.*, 1997), nonspatial avoidance task (Arendash *et al.*, 1995; Meyer *et al.*, 1994, 1997, 1998b), a 17-arm maze (Arendash *et al.*, 1995), and the Morris water maze (Meyer *et al.*, 1997). DMXBA also improves learning behavior as evidenced by enhanced performance during eye blink classical conditioning acquisition (Woodruff-Pak, 2003; Woodruff-Pak *et al.*, 1994) and performance in the Lashley III maze

(Arendash *et al.*, 1995). Some of these beneficial effects may be mediated by enhancement of long-term potentiation in hippocampal cells, a process important in learning and memory formation (Hunter *et al.*, 1994). Finally, a mouse model of schizophrenia-like cognitive and deficit symptoms, "popping," induced by the administration of a *N*-methyl-D-aspartic acid receptor antagonist is reduced following the administration of anabaseine (Mastropaolo *et al.*, 2004).

Given the known role of $\alpha 7$ nicotinic receptors in auditory gating, these drug candidates have also been tested for their ability to reverse auditory gating in animal models. As hypothesized, subcutaneous administration of DMXBA normalizes auditory gating in DBA/2 mice (Stevens *et al.*, 1998). Furthermore, a second injection of DMXBA produces a similar enhancement of inhibition. This lack of tachyphylaxis may represent improved efficacy of DMXBA in normalizing auditory gating on a chronic basis (Stevens *et al.*, 1998, 1999). Intragastrically administered DMXBA also enhanced impaired auditory gating, demonstrating that the medication can be effectively administered on an oral basis and is still efficacious at normalizing impaired auditory gating (Simosky *et al.*, 2001). DMXBA failed in another more complex and strain-dependent model of sensory gating, prepulse inhibition (Olivier *et al.*, 2001; Schreiber *et al.*, 2002). Despite this lack of effect of $\alpha 7$ nicotinic receptor agonists on prepulse inhibition measures, the robust reversal of P50 auditory gating deficits in these animal models is very promising for a similar effect in studies of auditory gating and cognition in schizophrenia.

V. The Phase 1 Study of DMXBA in Schizophrenia

On the basis of the success of preclinical trials of $\alpha 7$ agonists in animal models of learning and memory and the safety of these drugs, DMXBA was initially evaluated in normal subjects with a planned development for the treatment of dementia of the Alzheimer's type. DMXBA was found to significantly improve simple reaction time, correct detection during digit vigilance, both word and picture recognition memory, and both immediate and delayed word recall. Additionally, DMXBA improved subject performance speed on a numeric and spatial working memory task. Improvement was seen at doses from 25 to 150 mg with minimal adverse events (Kitagawa *et al.*, 2003). Despite these promising results, further development of DMXBA was not pursued by Taiho Pharmaceuticals. However, following the correction of the P50 auditory gating deficit by nicotine in persons with schizophrenia, the evidence of the $\alpha 7$ nicotinic receptor's role in this gating deficit in animal studies, as well as the reversal of sensory gating abnormalities in an animal model by the $\alpha 7$ nicotinic receptor agonist DMXBA,

this drug was identified as a potential candidate in the treatment of cognitive dysfunction in schizophrenia (Martin *et al.*, 2004).

A second phase I trial of DMXBA has been conducted in persons with schizophrenia (Olincy *et al.*, 2006). During this 3-visit study, DMXBA was administered in a double-blind fashion to 12 persons with schizophrenia. Doses were either placebo, a 75-mg dose with a 37.5-mg follow-up dose, or a 150-mg dose with a 75-mg follow-up dose. Subjects then underwent P50 auditory gating as well as neurocognitive testing. DMXBA normalized the P50 ratio (effect size of 2.36) as well as the test wave amplitude (effect size 1.45), a more specific measure of inhibition (Fig. 1). These findings are an improvement over the study of nicotine on P50 auditory gating in relatives (effect size 0.86). DMXBA was also

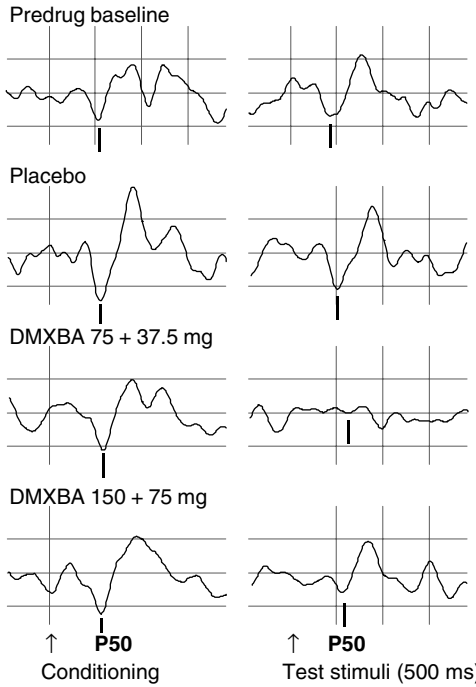

FIG. 1. Auditory-evoked responses of a subject with schizophrenia. Stimuli were a conditioning auditory stimulus and an identical test stimulus, delivered 500 ms apart. Inhibition of the test P50 response is increased by DMXBA administration, particularly during the lower dose (third row), compared to baseline and placebo responses above it. Arrows show the timing of the stimuli and vertical bars mark the location of the P50 wave in the tracings above. Positive polarity is downwards; vertical grid interval is 2 μV, and horizontal is 50 ms. This figure is reproduced with permission from Olincy *et al.* (2006). Proof-of-concept trial of an α7 nicotinic agonist in schizophrenia. *Arch. Gen. Psychiatry*, June 2006, **63,** 630–638; Copyright © 2006, American Medical Association. All rights reserved.

able to improve performance on both the Repeatable Battery for the Assessment of Neuropsychological Status' (RBANS) Total Scale score (effect size 1.8) as well as the Attention subscale (effect size 2.17; Fig. 2). These effect sizes are much larger than those seen for nicotine on the RBANS (0.6 and 0.25 for the Total scale score and Attention subscale score, respectively; Harris *et al.*, 2004) as well as for multiple other tests of the actions of nicotine on cognition in schizophrenia (effect sizes of 0.27–1.3; Dépatie *et al.*, 2002; Levin *et al.*, 1996; Myers *et al.*, 2004; Sacco *et al.*, 2005; Smith *et al.*, 2002). Furthermore, the effect sizes seen with DMXBA were also favorable when compared to the typical effect sizes of 0.2–0.5 for the effect of second generation antipsychotics on attentional and composite cognitive scores in persons with schizophrenia (Keefe *et al.*, 2004). The positive effects of DMXBA on sensory gating and cognition were not related to any changes in Brief Psychiatric Rating Scale scores and were therefore not due to changes in positive, negative, or anxiety-related symptoms.

These findings provide further evidence for a role of the nicotinic cholinergic system in the pathology of schizophrenia. Furthermore, specific $\alpha 7$ nicotinic cholinergic agonism is a therapeutic mechanism that provides hope for the

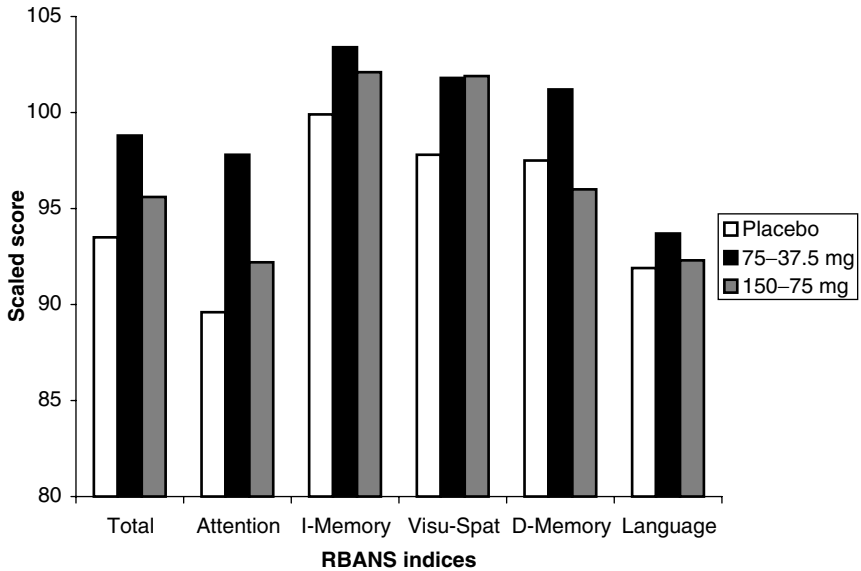

FIG. 2. Effects of DMXB A and placebo on the RBANS Total Scale score and its specific indices. I is immediate and D is delayed memory. This figure is reproduced with permission from Olincy *et al.* (2006). Proof-of-concept trial of an $\alpha 7$ nicotinic agonist in schizophrenia. *Arch. Gen. Psychiatry*, June 2006, **63,** 630–638; Copyright © 2006, American Medical Association. All rights reserved.

treatment of cognitive deficits in schizophrenia. As cognitive symptoms are more closely related to psychosocial dysfunction than traditional positive symptoms, such as hallucinations and delusions (Green, 1996), such a treatment could substantially increase the quality of life for persons with this devastating illness and reduce the financial burden of this disease.

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HISTAMINE AND SCHIZOPHRENIA

Jean-Michel Arrang

INSERM, U573, Unité de Neurobiologie et Pharmacologie Moléculaire
Centre Paul Broca, 2 ter rue d'Alésia, 75014 Paris, France

With the availability of an increased number of experimental tools, for example potent and brain-penetrating H_1-, H_2-, and H_3-receptor ligands and mutant mice lacking the histamine synthesis enzyme or the histamine receptors, the functional roles of histaminergic neurons in the brain have been considerably clarified during the recent years, particularly their major role in the control of arousal, cognition, and energy balance. Various approaches tend to establish the implication of histaminergic neurons in schizophrenia. A strong hyperactivity of histamine neurons is induced in rodent brain by administration of methamphetamine or NMDA-receptor antagonists. Histamine neuron activity is modulated by typical and atypical neuroleptics. H_3-receptor antagonists/inverse agonists display antipsychotic-like properties in animal models of the disease. Because of the limited predictability value of most animal models and the paucity of drugs affecting histaminergic transmission that were tried so far in human, the evidence remains therefore largely indirect, but supports a role of histamine neurons in schizophrenia.

INTERNATIONAL REVIEW OF NEUROBIOLOGY, VOL. 78
DOI: 10.1016/S0074-7742(06)78009-6

0074-7742/07 $35.00

I. Introduction

The idea that histamine may have a function as a neurotransmitter in brain emerged only slowly during the preceding century, essentially at the beginning of the 1970s (Schwartz, 1975), although it had been detected therein much earlier. The main landmarks in this history can be summarized as follows. The development of reliable and sensitive methods to assay the amine and its synthesizing enzyme (Schwartz *et al.*, 1970; Taylor and Snyder, 1971b) was instrumental in allowing to establish its localization in neurons, as well as its presence in a neural pathway traveling in the medial forebrain bundle as evidenced indirectly by lesion studies (Garbarg *et al.*, 1974). The turnover of the amine in cerebral neurons was found to be rapid and almost instantaneously modified by drugs like barbiturates or reserpine (Pollard *et al.*, 1973a,b; Taylor and Snyder, 1971a). The demonstration of depolarization-induced release and enhanced synthesis via calcium-dependent mechanisms (Atack and Carlsson, 1972; Taylor and Snyder, 1973; Verdiere *et al.*, 1975), the elucidation of inactivating metabolic pathways (Reilly and Schayer, 1970; Schwartz *et al.*, 1971), and the characterization in brain of the H_1 and H_2 receptors by biochemical and electrophysiological approaches (Baudry *et al.*, 1975; Haas and Bucher, 1975) completed by the mid-1970s the "picture" of histamine as a typical monoaminergic neurotransmitter. Even more, taking into account a variety of features of the system made available at this time, it was proposed that histaminergic neurons were critically involved in the control of arousal (Schwartz, 1977).

Nevertheless, it took nearly 10 years to develop reliable immunohistochemical tools that permitted to identify a tiny posterior hypothalamic area, the tuberomammillary nucleus, as the origin of the histaminergic pathways (Panula *et al.*, 1984; Watanabe *et al.*, 1983) and, thereby, fully convince the neurobiological community of their existence. At approximately the same time, the third histamine receptor was identified in our laboratory, which is almost exclusively present in brain where it controls the neurotransmitter release and synthesis (Arrang *et al.*, 1983) and developed the first selective and brain-penetrating ligands (Arrang *et al.*, 1987); these agents were used, thereafter, in hundreds of studies, to modify the activity of histaminergic neurons and, thereby, disclose their functions. These basic aspects, which were covered in detail in several comprehensive reviews (Brown *et al.*, 2001; Haas and Panula, 2003; Schwartz *et al.*, 1991; Watanabe and Yanai, 2001), will be briefly presented in the first part of the present chapter.

The evidence for the implication of histaminergic neurons in neuropsychiatric diseases remains largely indirect due to the poor predictability value of most animal models and the paucity of drugs affecting histaminergic transmission that were tried in these human diseases, so far. However, the changes in histamine

neuron activity, the modulation of the histaminergic system by neuroleptics, and the antipsychotic-like properties of H_3 receptor antagonists/inverse agonists support a role of histamine neurons in schizophrenia.

II. The Histaminergic Neuronal System

A. Organization

One decade after the first evidence by Garbarg *et al.* (1974) of an ascending histaminergic pathway obtained by lesions of the medial forebrain bundle, the exact localization of corresponding perikarya in the posterior hypothalamus was revealed immunohistochemically in the rat using antibodies against histamine (Panula *et al.*, 1984) or L-histidine decarboxylase (EC 4.1.1.22, HDC), the enzyme responsible for the one-step histamine formation in the brain (Watanabe *et al.*, 1984). Data on the distribution, morphology, and connections of histamine and HDC-immunoreactive neurons were comprehensively reviewed (Panula and Airaksinen, 1991; Panula *et al.*, 2000; Schwartz *et al.*, 1991; Tohyama *et al.*, 1991; Wouterlood and Steinbusch, 1991) and will be only summarized briefly here.

All known histaminergic perikarya constitute a continuous group of mainly magnocellular neurons located in the posterior hypothalamus and collectively named the tuberomammillary nucleus (Fig. 1). In the rat brain, the tuberomammillary nucleus consists of about 2000 histaminergic neurons (Ericson *et al.*, 1987) and can be subdivided into medial, ventral, and diffuse subgroups extending longitudinally from the caudal end of the hypothalamus to the midportion of the third ventricle. A similar organization was described in humans, except that histaminergic neurons are more numerous (~64,000) and occupy a larger proportion of the hypothalamus (Airaksinen *et al.*, 1991). Neurons expressing mRNAs for histidine decarboxylase were found by *in situ* hybridization in the tuberomammillary nucleus, but not in any other brain area (Bayliss *et al.*, 1990). The histaminergic neurons are characterized by the presence of an unusually large variety of markers for other neurotransmitter systems. Most, if not all, contain γ-aminobutyric acid (GABA; Airaksinen *et al.*, 1992; Ericson *et al.*, 1991b), adenosine deaminase, a cytoplasmic enzyme possibly involved in adenosine inactivation (Patel *et al.*, 1986; Senba *et al.*, 1985), and a splice variant of choline acetyltransferase (Kanayama *et al.*, 2003). Some histaminergic neurons also express several neuropeptides but these colocalizations are observed in various proportions and display strong species differences (Airaksinen *et al.*, 1992; Trottier *et al.*, 2002).

In analogy with other monoaminergic neurons, histaminergic neurons constitute long and highly divergent systems projecting in a diffused manner to many cerebral areas (Panula and Airaksinen, 1991; Tohyama *et al.*, 1991; Wouterlood

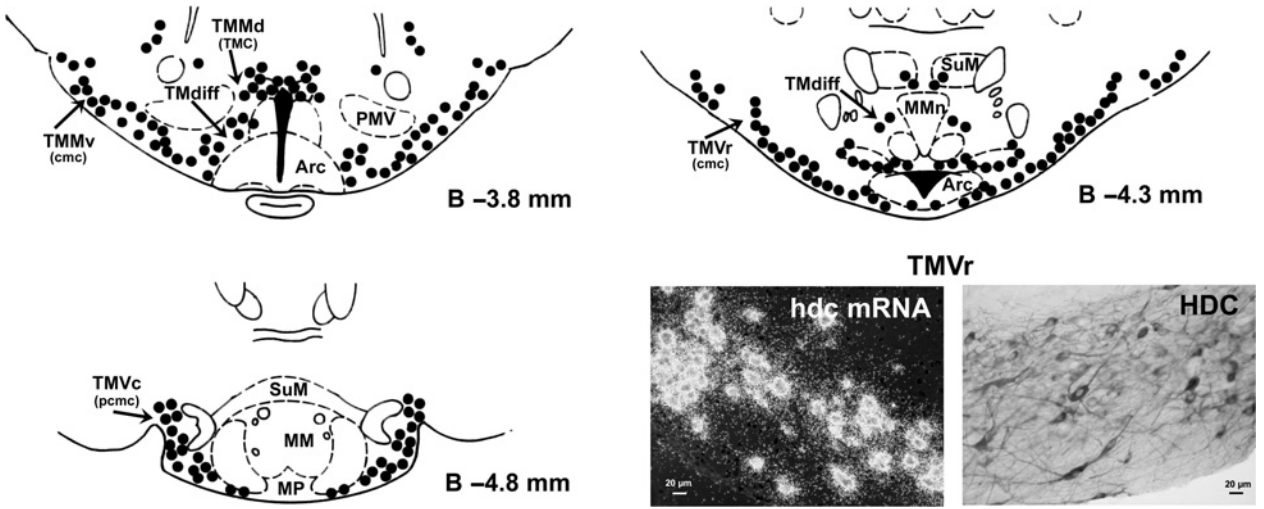


FIG. 1. Localization of histaminergic perikarya in the tuberomammillary nucleus. Histaminergic perikarya are represented by closed circles on frontal sections at indicated levels of caudal hypothalamus. Histidine decarboxylase (HDC) mRNA expression (left) and immunoreactivity (right) is shown in the ventral tuberomammillary subgroup (rostral part). Abbreviations: Arc, arcuate nucleus; cmc, caudal magnocellular nucleus; MM, medial mammillary nucleus medial part; MMn, medial mammillary nucleus median part; MP, medial mammillary nucleus posterior part; pcmc, posterior caudal magnocellular nucleus; PMV, premammillary nucleus ventral part; SuM, supramammillary nucleus; TMC, tuberal magnocellular nucleus; TMdiff, tuberomammillary nucleus diffuse part; TMMd, medial tuberomammillary subgroup dorsal part; TMMv, medial tuberomammillary subgroup ventral part; TMVc, ventral tuberomammillary subgroup caudal part; TMVr, ventral tuberomammillary subgroup rostral part.

and Steinbusch, 1991; Fig. 2). Immunoreactive, mostly unmyelinated, varicose or nonvaricose fibers are detected in almost all cerebral regions, particularly limbic structures. It was confirmed that individual neurons project to widely divergent areas, mainly in an ipsilateral fashion. Major areas of termination of these long ascending fibers arising from the tuberomammillary nucleus are all layers of the cerebral cortex, the olfactory bulb, the hippocampus, the nucleus accumbens, the globus pallidus, the thalamus, the amygdaloid complex, and many hypothalamic nuclei. A histaminergic neuronal system reminiscent of that described in rodents is present in the monkey and human brain with, for example, a dense network of fibers present in various cortical areas or thalamic nuclei (Jin *et al.*, 2002; Panula *et al.*, 1990; Wilson *et al.*, 1999).

Several anterograde and retrograde tracing studies established the existence of afferent connections to the histaminergic perikarya, namely from the infralimbic cortex, the septum-diagonal band complex, the preoptic region, the hypothalamus, and the hippocampal area (subiculum; Ericson *et al.*, 1991a; Wouterlood and Steinbusch, 1991). Sleep-active GABAergic neurons in the ventrolateral preoptic nucleus (VLPO) provide a major input to the tuberomammillary nucleus (Sherin *et al.*, 1996, 1998). The contacts between these two systems are reciprocal because the VLPO is densely innervated by histaminergic fibers (Chou *et al.*, 2002). Histaminergic neurons also receive very dense orexin innervation originating from the lateral hypothalamus (Chemelli *et al.*, 1999). Again, the relationships between the orexin and histamine systems seem to be reciprocal because the orexin neurons are heavily innervated by histaminergic axons (Eriksson *et al.*, 2001a). Supporting their role in the regulation of food intake, histaminergic neurons are densely innervated by nerve fiber varicosities immunoreactive for amylin and α-melanocyte stimulating hormone, two anorexigenic peptides (D'Este *et al.*, 2001; Fekete and Liposits, 2003). Projections from the brainstem to the tuberomammillary nucleus have also been demonstrated. Monoaminergic inputs to the tuberomammillary nucleus originate mainly from the medulla oblongata and from the raphe nuclei, with a lower innervation originating from the locus coeruleus, the ventral tegmental area, and the substantia nigra (Ericson *et al.*, 1989; Sakai *et al.*, 1990).

B. Metabolism of Histamine

Histamine biosynthesis in the brain involves two steps: transport of the precursor L-histidine (His) into the cell and its subsequent decarboxylation by HDC (Schwartz *et al.*, 1991). The human HDC gene is composed of 12 exons and its transcripts are alternatively spliced but only the 2.4-kb mRNA, which is predominant in human brain, encodes functional HDC (Yatsunami *et al.*, 1994). The native HDC is a pyridoxal phosphate-dependent enzyme under a homodimeric form

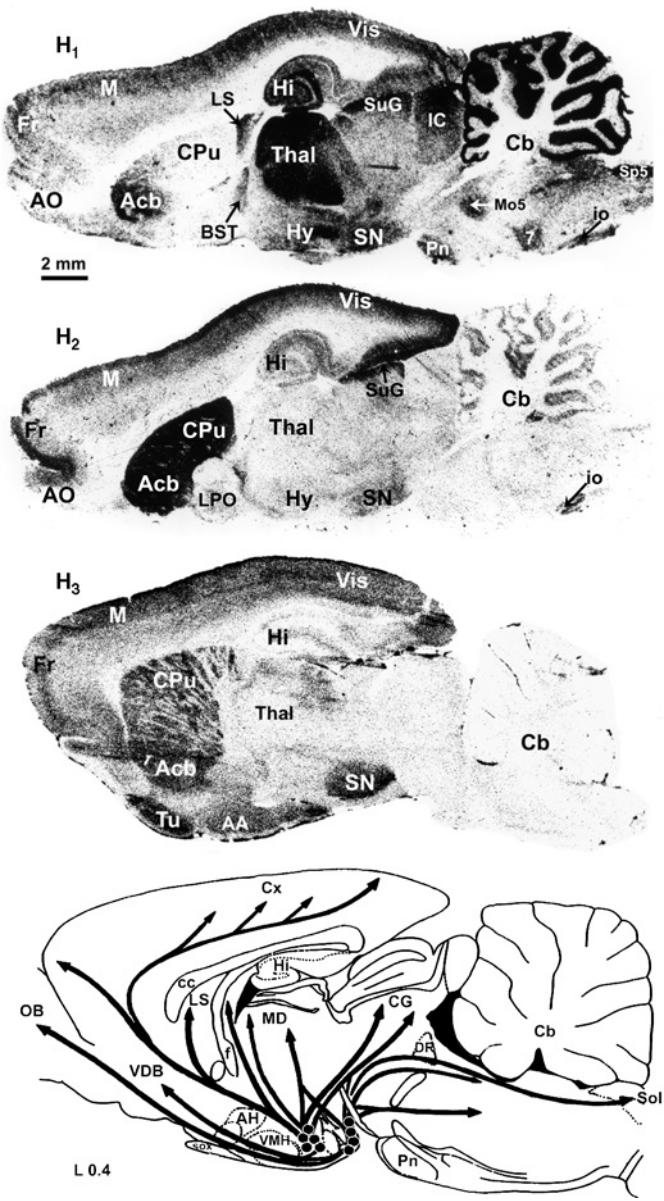


FIG. 2. Autoradiographic localization of histamine receptors and disposition of main histaminergic pathways on sagittal brain sections. H_1 and H_2 receptors were visualized on guinea pig brain sections using [^{125}I]iodobolpyramine and [^{125}I]iodoaminopotentidine, respectively. H_3 receptors were visualized on rat brain sections using [^{125}I]iodoproxyfan. Histaminergic pathways (arrows) were

constituted of two subunits of a 54-kDa isoform and mainly found in the cytoplasm of histaminergic neurons. The regional distribution of brain HDC activity is consistent with data derived from immunohistochemistry, with the highest activity being found in the hypothalamus, the lowest levels in the cerebellum and intermediate activity in telencephalic areas (Schwartz *et al.*, 1970, 1991). HDC-knockout mice provide a suitable model to investigate the involvement of histaminergic neurons in the regulation of fundamental functions such as sleep-wake control (Parmentier *et al.*, 2002), energy homeostasis (Fülop *et al.*, 2003), learning (Dere *et al.*, 2003), seizure development (Chen *et al.*, 2003b), motor and emotional behaviors (Dere *et al.*, 2004; Iwabuchi *et al.*, 2004), or circadian rhythms (Abe *et al.*, 2004).

In neurons, the newly synthesized histamine is transported to vesicles by the vesicular monoamine transporter 2 (VMAT2) for which it displays high affinity (Peter *et al.*, 1994). Although histaminergic neurons constitute the major localization of HDC, at least two other types of histamine-producing cells, mast cells and microglial cells, have been reported by lesion, biochemical, and histochemical studies. Although they are rather scarce in the brain, mast cells are generally abundant in leptomeninges and also occur in the parenchyma of various brain areas, such as thalamus, where they are mainly distributed along cerebral vessels (Schwartz *et al.*, 1991). Microglial cells belong to the monocyte/macrophage lineage and also contain both HDC activity and mRNAs (Katoh *et al.*, 2001).

Brain histamine is metabolized via transmethylation into *tele*-methylhistamine (*tele*-MeHA) catalyzed by histamine *N*-methyltransferase (HMT, EC 2.1.1.8). *In vivo* inhibition of HMT increases neuronal histamine release, confirming that this enzyme plays a critical role in histamine inactivation (Itoh *et al.*, 1991). Its levels are decreased in Down syndrome and increased in Pick's disease (Kim *et al.*, 2002), and the drug tacrine, which is used in long-term palliative treatment of Alzheimer's disease, inhibits HMT, even more potently than acetylcholinesterase (Morisset *et al.*, 1996). HMT-like immunoreactivity within the CNS was found in the cytosol of a variety of neurons and in vascular walls, whereas astrocytes were not stained (Nishibori *et al.*, 2000). How extracellular histamine is transported

represented on a sagittal section of rat brain. Abbreviations: 7, facial nucleus; AA, amygdaloid area; Acb, accumbens nucleus; AH, anterior hypothalamic area; AO, anterior olfactory nuclei; BST, bed nucleus of the stria terminalis; Cb, cerebellum; cc, corpus callosum; CG, central gray; CPu, caudate putamen; Cx, cortex; DR, dorsal raphe nucleus; f, fornix; Fr, frontal cortex; Hi, hippocampus; Hy, hypothalamus; IC, inferior colliculus; io, inferior olive; LPO, lateral preoptic area; LS, lateral septum; M, motor cortex; MD, mediodorsal thalamic nucleus; Mo5, motor trigeminal nucleus; OB, olfactory bulb; Pn, pontine nucleus; SN, substantia nigra; Sol, nucleus of the solitary tract; sox, supraoptic decussation; Sp5, spinal trigeminal nucleus; SuG, superficial gray layer of superior colliculus; Thal, thalamus; Tu, olfactory tubercle; VDB, nucleus of the vertical limb diagonal band; Vis, visual cortex; VMH, ventromedial hypothalamic nucleus.

into these HMT-containing cells is still unclear. In contrast with other monoaminergic systems, no clear evidence for a high-affinity uptake system for histamine could be found (Schwartz *et al.*, 1991). There is, however, some evidence that histamine could be transported by a low-affinity, low-specificity, high-capacity system (Jonker and Schinkel, 2004).

C. Histamine Receptors

In the brain, the effects of histamine are mediated by three histamine receptor subtypes (H_1, H_2, and H_3), which have been defined by means of functional assays followed by design of selective agonists and antagonists and cloning of their genes (Hill *et al.*, 1997; Schwartz and Arrang, 2002). All three belong to the superfamily of receptors with seven transmembrane domains and coupled to guanylnucleotide-sensitive G-proteins (Table I).

1. *The Histamine H_1 Receptor*

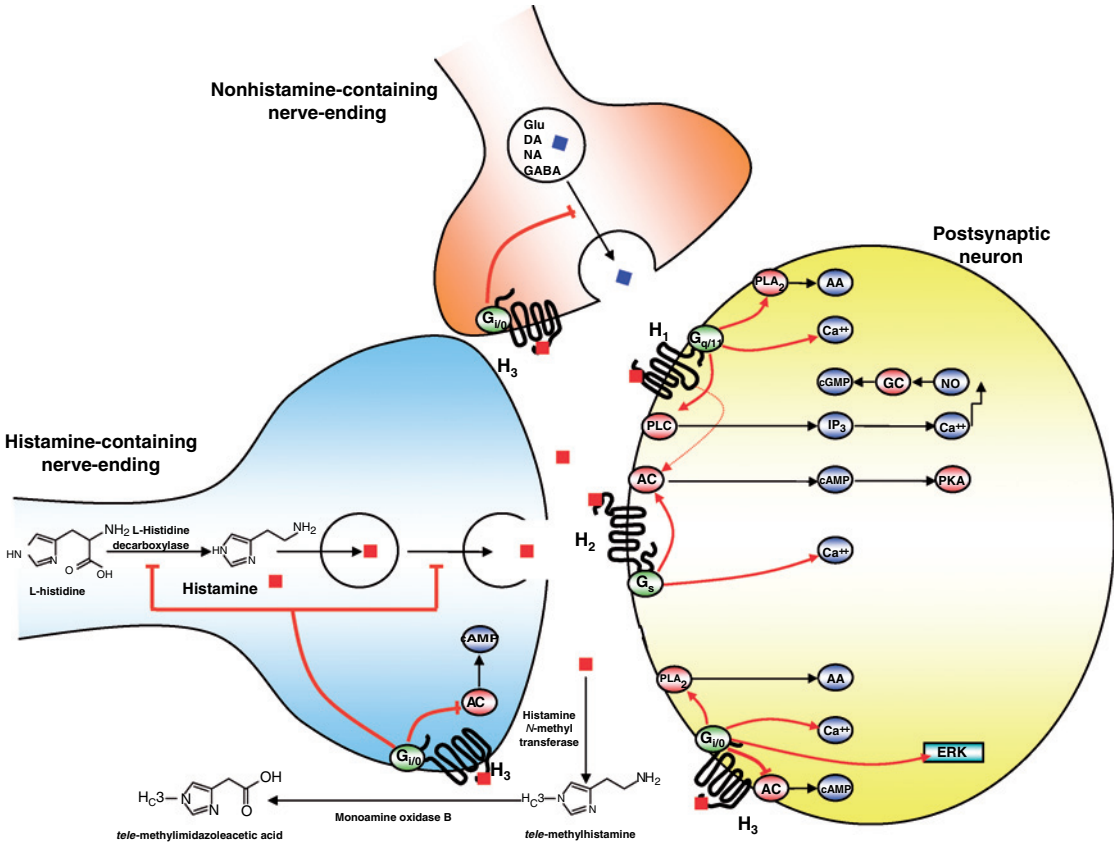
The H_1 receptor was initially defined in functional assays (e.g., smooth muscle contraction) and the design of potent antagonists, the so-called "antihistamines" (e.g., mepyramine), most of which display prominent sedative properties. It was first cloned from cow by expression cloning (Yamashita *et al.*, 1991), and subsequently, from a variety of species, including man (Hill *et al.*, 1997). The human gene contains an intron in the 5′-flanking untranslated region, close to the translation initiation codon, but the translated region is intronless (De Backer *et al.*, 1998). The histamine H_1 receptor produces its intracellular effects via the activation of $G_{q/11}$ proteins (Hill *et al.*, 1997; Leopoldt *et al.*, 1997; Fig. 3). In brain tissues and various cell systems (Leurs *et al.*, 1994; Schwartz *et al.*, 1991), H_1 receptor activation leads to stimulation of phospholipase Cβ and inositol phosphate release. The subsequent mobilization of Ca^{2+} from intracellular stores followed by an influx of extracellular Ca^{2+} induces an increase in intracellular Ca^{2+} levels. This process is presumably responsible for the activation of various Ca^{2+}-dependent pathways by the recombinant or native H_1 receptor, such as potentiation of cAMP accumulation, cGMP accumulation, arachidonic acid release, and glycogenolysis (Leurs *et al.*, 1994; Schwartz *et al.*, 1991). The H_1 receptor mediates mainly excitatory responses in brain, leading to a depolarization and/or an increase in firing frequency in many neurons (Brown *et al.*, 2001; Haas and Panula, 2003).

Biochemical and localization studies of the H_1 receptor were made feasible with the design of reversible and irreversible radiolabeled probes such as [^{3}H] mepyramine and [^{125}I]iodobolpyramine (Garbarg *et al.*, 1992; Pollard and Bouthenet, 1992). The distribution in the brain of H_1 receptor-binding sites is consistent with a predominant neuronal localization of H_1 receptors. They are abundant in guinea pig thalamus, hypothalamic nuclei (e.g., ventromedial

TABLE I
PROPERTIES OF FOUR HISTAMINE RECEPTOR SUBTYPES

	H_1	H_2	H_3	H_4
Coding sequence	491 a.a. (b) 488 a.a. (gp) 486 a.a. (r)	358 a.a. (r) 359 a.a. (d, h, gp)	445 a.a. (h) Shorter variants (h, r, m, gp)	390 a.a. (h) 389 a.a. (gp) 391 a.a. (m, r)
Chromosome localization	3p25	5	20qTEL	18q11.2
Highest brain densities	Thalamus Cerebellum Hippocampus	Striatum Cerebral cortex Amygdala	Striatum Frontal cortex Substantia nigra	Very low density
Autoreceptor	No	No	Yes	No
Affinity for histamine	Micromolar	Micromolar	Nanomolar	Nanomolar
Characteristic agonists	Histaprodifen	Impromidine	R-α-methylhistamine	4-methylhistamine
Characteristic antagonists	Mepyramine	Cimetidine	Thioperamide	JNJ 7777120
Radioligands	[^{3}H]Mepyramine [^{125}I]Iodobolpyramine	[^{3}H]Tiotidine [^{125}I]Iodoamino-potentidine	[^{3}H] R-α-methylhistamine [^{125}I]Iodoproxyfan	[^{3}H]Histamine
Second messengers	Inositol phosphates (+) Ca^{2+} (+) Arachidonic acid (+) cAMP (potentiation)	cAMP (+) Ca^{2+} (+)	cAMP (−) Inositol phosphates (−) Arachidonic acid (+) Ca^{2+} (−)	cAMP (−)

a.a., amino acid.


Nonhistamine-containing nerve-ending
Glu
DA
NA
GABA
Postsynaptic neuron
Histamine-containing nerve-ending
L-Histidine decarboxylase
L-histidine
Histamine
Histamine N-methyl transferase
Monoamine oxidase B
tele-methylimidazoleacetic acid
tele-methylhistamine
PLA2
AA
Ca++
cGMP
GC
NO
PLC
IP3
cAMP
PKA
ERK
AC
H1
H2
H3
Gi/0
Gq/11
Gs

nuclei), nucleus accumbens, amygdaloid nuclei, and frontal cortex but not in caudate-putamen (Pollard and Bouthenet, 1992; Fig. 2). In the human brain, binding sites are more abundant in the neostriatum than in the guinea pig (Martinez-Mir *et al.*, 1990), and, in agreement with the H_1 receptor-mediated modulation of thalamocortical functions by histamine, H_1 receptor-binding sites and mRNAs are abundant in the human thalamus and prefrontal cortex (Jin and Panula, 2005; Jin *et al.*, 2002).

Blockade of H_1 receptors in brain is presumably involved in the sedative, pro-obesity and proconvulsant properties of many drugs displaying high H_1 receptor affinity. The behavioral data obtained with H_1 receptor knockout mice largely support a role of H_1 receptors in arousal and cognition (Huang *et al.*, 2001; Inoue *et al.*, 1996; Lin *et al.*, 2002), anxiety and aggressive behavior (Yanai *et al.*, 1998), nociception (Mobarakeh *et al.*, 2000; Sakurada *et al.*, 2004), anticonvulsant action (Chen *et al.*, 2003b; Hirai *et al.*, 2004), and regulation of food intake and body weight (Masaki *et al.*, 2004; Morimoto *et al.*, 1999).

2. *The Histamine H_2 Receptor*

Studies of the molecular properties of the H_2 receptor have been greatly facilitated in the 1990s by the design of its first potent and selective radioligand, [^{125}I]iodoaminopotentidine (Ruat *et al.*, 1990; Traiffort *et al.*, 1992), as well as by the cloning of its gene in various species including man (Gantz *et al.*, 1991a,b; Hill *et al.*, 1997). The recombinant or native H_2 receptor is coupled to G_s proteins and mediates activation of adenylyl cyclase with subsequent increases in cAMP formation and protein kinase A activation (Green *et al.*, 1977; Hegstrand *et al.*, 1976; Schwartz *et al.*, 1991; Fig. 3). As the H_1 receptor, the H_2 receptor usually mediates excitatory responses in neurons (Brown *et al.*, 2001; Haas and Panula, 2003). Its distribution in the brain is consistent with a predominant neuronal localization. Autoradiographic localization of the H_2 receptor using [^{125}I]iodoaminopotentidine in the guinea pig (Vizuete *et al.*, 1997), monkey, and human brain (Honrubia *et al.*, 2000; Martinez-Mir *et al.*, 1990; Traiffort *et al.*, 1992) shows it distributed heterogeneously (Fig. 2). The H_2 receptor is found in most areas of the cerebral cortex. The caudate putamen, ventral striatal complex and amygdaloid nuclei (bed nucleus of the stria terminalis) are among the richest brain areas. The distribution of the mRNAs is generally in agreement with that of the corresponding binding sites. In the striatum, the absence of mRNAs in the

FIG. 3. Histamine metabolism and signaling in brain. Abbreviations: AA, arachidonic acid; AC, adenylate cyclase; DA, dopamine; ERK, extracellular signal-related kinase; GABA, γ-aminobutyric acid; GC, guanylate cyclase; Glu, glutamate; IP_3, inositol-1,4,5-triphosphate; NA, noradrenaline; PKA, protein kinase A; PLA_2, phospholipase A_2; PLC, phospholipase C; NO, nitric oxide.

substantia nigra together with the loss of binding sites in Huntington's disease (Martinez-Mir *et al.*, 1993) indicate that H_2 receptors are expressed by intrinsic neurons. The brain-penetrating H_2 receptor antagonist, zolantidine, has been used to investigate the involvement of H_2 receptors in various neurochemical and behavioral responses (Mori *et al.*, 2004; Nalwalk *et al.*, 1995). A number of tricyclic antidepressants are very potent inhibitors of the H_2 receptor-linked adenylyl cyclase on brain membranes (Green and Maayani, 1977; Kanof and Greengard, 1978), but not on intact cell preparations (Dam Trung Tuong *et al.*, 1980). In addition, the idea that antidepressants derive their clinical efficacy from blockade of cerebral H_2 receptors seems unlikely because such a blockade was not observed after chronic treatments (Nowak *et al.*, 1983).

3. *The Histamine H_3 Receptor*

The H_3 receptor was initially detected and identified by traditional pharmacological approaches as an autoreceptor controlling histamine synthesis and release in the rat and human brain (Arrang *et al.*, 1983, 1987, 1988). The cloning of its cDNAs in various species including human and rat confirmed that the H_3 receptor is coupled to G_i/G_o proteins (Drutel *et al.*, 2001; Lovenberg *et al.*, 1999; Morisset *et al.*, 2000). In various cell lines, activation of the recombinant H_3 receptor inhibits adenylate cyclase, activates phospholipase A_2, and activates the ERK signaling pathway (Fig. 3). Although a direct inhibition of adenylate cyclase could not be observed in various brain regions (Garbarg *et al.*, 1989; Schlicker *et al.*, 1994a), H_3 autoreceptors modulate histamine synthesis through the cAMP pathway (Gomez-Ramirez *et al.*, 2002; Torrent *et al.*, 2005) and H_3 receptor activation inhibits dopamine D_1 receptor-mediated cAMP formation in the rat striatum (Sanchez-Lemus and Arias-Montano, 2004). Recombinant H_3 receptors display a high level of constitutive (or spontaneous) activity and most antagonists act in fact as inverse agonists on various responses (Esbenshade *et al.*, 2005; Morisset *et al.*, 2000). Consistent with the physiological relevance of the process, constitutive activity of native H_3 receptors is detected in rodent brain (Morisset *et al.*, 2000; Rouleau *et al.*, 2002). Inverse agonists at H_3 receptors enhance histamine neuron activity by abrogating the brake triggered by constitutive activity of H_3 autoreceptors (Gbahou *et al.*, 2003; Morisset *et al.*, 2000) and are, therefore, important tools to delineate the functions of histaminergic neurons. The recombinant human H_3 receptors expressed at physiological densities also display constitutive activity, suggesting it is present in human brain (Rouleau *et al.*, 2002).

After its characterization as an autoreceptor present on histamine neurons, the H_3 receptor was shown to inhibit presynaptically the release of other monoamines in brain. H_3 receptors inhibit the *in vitro* release of various neurotransmitters, including histamine itself, noradrenaline, serotonin, dopamine, glutamate, GABA, and

tachykinins (Schlicker *et al.*, 1994b; Schwartz and Arrang, 2002). This presynaptic inhibition presumably results from a direct G-protein-mediated blockade of voltage-gated calcium channels (Brown and Haas, 1999; Jang *et al.*, 2001; Silver *et al.*, 2002). In brain slices or isolated neurons, somatodendritic H_3 autoreceptors also inhibit the firing rate of histaminergic neurons by inhibiting multiple high-threshold calcium channels (Stevens *et al.*, 2001; Takeshita *et al.*, 1998).

The inhibition mediated by H_3 autoreceptors is now well established as a major control mechanism for the activity and functions of histaminergic neurons. However, the physiological role of the H_3 receptors present on other neuronal populations remains largely unknown. In the striatum, H_3 heteroreceptors inhibit dopamine synthesis (Molina-Hernandez *et al.*, 2000) and release (Schlicker *et al.*, 1993) but do not regulate dopamine neuron activity *in vivo* under basal conditions (Imaizumi and Onodera, 1993; Miyazaki *et al.*, 1997; Oishi *et al.*, 1990b). In the striatum, dentate gyrus, and amygdala, H_3 receptor activation inhibits glutamatergic transmission *in vitro* (Brown and Reymann, 1996; Doreulee *et al.*, 2001; Jiang *et al.*, 2005; Molina-Hernandez *et al.*, 2001), but the standard antagonist/inverse agonist thioperamide does not increase synaptic potentials in the freely moving rat (Manahan-Vaughan *et al.*, 1998). H_3 receptors inhibit GABA release in rat striatum, substantia nigra, and hypothalamus (Arias-Montano *et al.*, 2001; Garcia *et al.*, 1997; Jang *et al.*, 2001).

A detailed autoradiographic mapping of the rat H_3 receptor was achieved with the selective antagonist [^{125}I]iodoproxyfan (Pillot *et al.*, 2002a; Fig. 2). The comparison with the distribution of H_3 receptor mRNAs provides evidence for the presence of H_3 receptors on many neuronal perikarya, dendrites, and projections. The highest receptor densities are found in the cerebral cortex, basal ganglia, olfactory tubercles, amygdala, and tuberomammillary nucleus. Receptor densities are particularly high in the striatum where lesions indicated that most H_3 receptors are present on projection neurons (Anichtchik *et al.*, 2000a; Cumming *et al.*, 1991; Pollard *et al.*, 1993; Ryu *et al.*, 1994a,b, 1995). In agreement, high densities of H_3 receptor mRNAs are also found in the striatum from rat, guinea pig, and human (Anichtchik *et al.*, 2001; Pillot *et al.*, 2002a; Tardivel-Lacombe *et al.*, 2000). H_3 receptors present on striatonigral neurons account for the dense binding in the substantia nigra pars reticulata (Ryu *et al.*, 1996). H_3 receptors are also expressed in striatopallidal projection neurons (Pillot *et al.*, 2002b) and account for the dense binding in the external globus pallidus in rat (Pillot *et al.*, 2002a) and human (Anichtchik *et al.*, 2001; Martinez-Mir *et al.*, 1990). This expression in the external pallidum is increased in Parkinson's disease (Anichtchik *et al.*, 2001) and dramatically reduced in Huntington's disease (Goodchild *et al.*, 1999). H_3 receptor functions have primarily been studied with standard agonists and antagonists/inverse agonists and with mice lacking H_3 receptors (Koyama *et al.*, 2003; Rizk *et al.*, 2004; Takahashi *et al.*, 2002; Toyota *et al.*, 2002).

4. *The Histamine H_4 Receptor*

Several groups cloned an additional histamine receptor subtype, termed H_4, via *in silico* analysis of human genomic databases (Hough, 2001). Its gene structure, coding sequence, and pharmacology are clearly related to the H_3 receptor. Only few selective ligands have so far been designed for this novel receptor (Gbahou *et al.*, 2006; Lim *et al.*, 2005; Thurmond *et al.*, 2004) and antagonists mainly display anti-inflammatory properties (de Esch *et al.*, 2005). Its expression in the brain is very low, if any, and was detected only in some studies (Coge *et al.*, 2001; Liu *et al.*, 2001a). Its highest level of expression is observed in hematopoietic tissues and cells (bone marrow, spleen, leucocytes; Hough, 2001).

D. Histaminergic Neuron Activity and Its Control

The autoreceptor-regulated modulation of histamine synthesis in, and release from, brain neurons is well documented (Schwartz *et al.*, 1991). It was initially evidenced in brain slices or synaptosomes (Arrang *et al.*, 1985) after labeling the endogenous pool of histamine using the [^{3}H]-precursor (Verdiere *et al.*, 1975). Exogenous histamine decreases the release and formation of [^{3}H]histamine induced by depolarization, and analysis of these responses led to the pharmacological definition of H_3 receptors. The inhibition mediated by the H_3 autoreceptor constitutes a major regulatory mechanism for histaminergic neuron activity under physiological conditions (Arrang *et al.*, 1987; Schwartz *et al.*, 1991). Administration of selective H_3 receptor agonists reduces histamine turnover and release *in vivo* (Garbarg *et al.*, 1989; Itoh *et al.*, 1992). In contrast, H_3 receptor antagonists/inverse agonists enhance histamine turnover and release *in vivo*, indicating that autoreceptors are tonically activated (Itoh *et al.*, 1991; Ligneau *et al.*, 1998; Mochizuki *et al.*, 1991; Morisset *et al.*, 2000).

In vivo, both neurochemical and electrophysiological studies indicate that the activity of histaminergic neurons is maximal during arousal (Schwartz *et al.*, 1991). Neurons identified as histaminergic neurons exhibit a circadian rhythm of their firing rate, highest during waking and falling silent during deep slow-wave or paradoxical sleep (hence referred to as "waking-on" or "REM-off" neurons; Steininger *et al.*, 1999; Vanni-Mercier *et al.*, 2003). An important determinant of this circadian rhythm of tuberomammillary histaminergic neuron activity is the GABAergic inhibitory input from the VLPO which is activated during sleep (Sherin *et al.*, 1996, 1998; Yang and Hatton, 1997). GABAergic inhibitory postsynaptic potentials are mediated by $GABA_A$ receptors located on histaminergic neurons (Stevens *et al.*, 1999). Histaminergic neurons contain GABA (Airaksinen *et al.*, 1992; Ericson *et al.*, 1991b), but to what extent these receptors play an autoinhibitory role is unclear. Histamine turnover in the brain is rapidly reduced after administration of GABAergic sedative drugs such as barbiturates and

benzodiazepines (Oishi *et al.*, 1986; Pollard *et al.*, 1974), presumably as a result of their interaction with these $GABA_A$ receptors. *In vivo* microdialysis shows that endogenous GABA as well as systemic administration of muscimol, pentobarbital, diazepam, and halothane inhibits histamine release in the rat brain (Chikai *et al.*, 1993; Mammoto *et al.*, 1997; Okakura-Mochizuki *et al.*, 1996). $GABA_A$ receptors present in the tuberomammillary nucleus also play a key role in the sedative component of anesthesia. Microinjection of muscimol in the tuberomammillary nucleus produces sedation in rats and cats (Lin *et al.*, 1989; Nelson *et al.*, 2002), and systemic administration of muscimol, propofol, or pentobarbital decreases fos expression in the tuberomammillary nucleus (Nelson *et al.*, 2002).

Orexins directly excite the histaminergic neurons *in vitro* (Bayer *et al.*, 2001; Eriksson *et al.*, 2001a). Most histamine neurons express mRNAs and immunoreactivity for both orexin receptors (Eriksson *et al.*, 2001a; Marcus *et al.*, 2001), but the orexin 2 receptor seems mainly involved (Willie *et al.*, 2003). Orexins released from neurons emanating from the lateral hypothalamus enhance histamine neuron activity. Orexin levels are not altered by circadian time but their arousal effect depends on activation of histaminergic neurons (Huang *et al.*, 2001; Shigemoto *et al.*, 2004). In agreement, an altered histamine level was reported in orexin receptor-2-mutated dogs, an animal model of narcolepsy (Nishino *et al.*, 2001).

Several other systems activate or inhibit histamine neurons. Serotonin depolarizes histamine neurons and increases their firing rate *in vitro* by activation of $5\text{-}HT_{2C}$ receptors and an NCX1 Na^+/Ca^{2+}exchanger (Eriksson *et al.*, 2001b). Several types of serotonergic receptors are likely to modulate histamine neuron activity *in vivo* (Laitinen *et al.*, 1995a; Morisset *et al.*, 1999; Oishi *et al.*, 1992). Ghrelin, a potent orexigenic peptide, activates histamine neurons by inhibiting G-protein–coupled inward rectifier K^+ (GIRK) channels (Bajic *et al.*, 2004; Nakazato *et al.*, 2001). Intracellular recordings from rat hypothalamic slices indicate that morphine increases the firing of histaminergic neurons (Eriksson *et al.*, 2000), and histamine release in mouse cerebral cortex is enhanced by stimulation of κ-opioid receptors (Itoh *et al.*, 1988). Morphine and μ-opioid receptor agonists (Chikai *et al.*, 1994; Itoh *et al.*, 1988) enhance histamine release and turnover *in vivo*.

Inhibitory actions on histaminergic neurons have also been found. Besides GABA, nociceptin inhibits the firing of histaminergic neurons by activating GIRK channels (Eriksson *et al.*, 2000). [^{3}H]Histamine synthesis and release are inhibited in various brain regions by stimulation of not only autoreceptors but also α_2-adrenergic receptors, M_1-muscarinic receptors and κ-opioid receptors (Schwartz *et al.*, 1991). Since these regulations are also observed with synaptosomes, all these receptors presumably represent true presynaptic heteroreceptors. Agents inhibiting histamine release *in vitro* via stimulation of presynaptic α_2-adrenergic receptors reduce histamine release and turnover *in vivo* (Gulat-Marnay *et al.*, 1989a; Prast *et al.*, 1991). A similar inhibition is induced by activation of muscarinic heteroreceptors

(Gulat-Marnay *et al.*, 1989b; Oishi *et al.*, 1990a; Prast *et al.*, 1994). Whether these heteroreceptors are tonically activated under basal conditions remains unclear: systemic administration of antagonists of these receptors does not enhance histamine turnover, but *in vivo* microdialysis studies show that their local perfusion increases histamine release (Laitinen *et al.*, 1995b; Prast *et al.*, 1994).

An increase of *tele*-methylhistamine levels with age has been reported in human cerebrospinal fluid (Prell *et al.*, 1990). Changes in histamine neuron activity also occur in various neuropsychiatric diseases. *tele*-Methylhistamine levels are increased in the cerebrospinal fluid of schizophrenic patients (Prell *et al.*, 1995). Histamine levels are unaffected in the brain of MPTP-treated mice (Cumming *et al.*, 1989) but increased in the brain of patients with Parkinson's disease, where they are associated with a strong increase in histaminergic innervation in the substantia nigra (Anichtchik *et al.*, 2000b; Rinne *et al.*, 2002). A reduced number of histamine neurons (Nakamura *et al.*, 1993) and a significant decrease of HDC activity and histamine levels in the hypothalamus, hippocampus, and cortex (Panula *et al.*, 1998; Schneider *et al.*, 1997) have been found in Alzheimer's brains. Similar deficits have been reported in frontal cortex of patients with Down syndrome (Schneider *et al.*, 1997).

E. Physiological Roles of Histaminergic Neurons

Owing to the use of an increased number of experimental tools, for example, histamine H_3 receptor antagonists to activate histaminergic neurons (Arrang *et al.*, 1987), α-fluoromethylhistidine to block brain histamine synthesis (Garbarg *et al.*, 1980), mutant mice lacking L-histidine decarboxylase (Parmentier *et al.*, 2002) or the histamine H_1 receptor (Inoue *et al.*, 1996), the functional role of histaminergic neurons has been considerably clarified during the recent years.

Since our initial proposal in 1977 (Schwartz, 1977), a large variety of studies are now available to strengthen the view that the histaminergic system is one of the major neuronal systems controlling cortical activation and wakefulness (Lin, 2000). In agreement, ablation of these neurons, inhibition of histamine synthesis, release or action via the H_1 receptor all decrease wakefulness and increase deep slow-wave sleep; conversely, inhibition of histamine methylation or facilitation of histamine release via H_3 autoreceptor blockade all increase arousal. The major part played by the H_1 receptor in histamine-induced arousal, confirmed in mutant mice lacking this receptor (Inoue *et al.*, 1996), accounts for the sedating effects of the first generation of "antihistamines," that is, antagonists which easily enter the brain and are still ingredients of over-the-counter sleeping pills (Sangalli, 1997; Tashiro *et al.*, 2002).

The idea that activation of histaminergic neurons might improve cognitive performances is consistent with their projections to brain areas such as the

prefrontal and cingulate cortices or hippocampus, their projections to cholinergic perikarya, their excitatory influences therein, and their positive role in wakefulness. The procognitive and proattentional roles of histaminergic neuron activation were largely established in behavioral studies in rodents using thioperamide or other H_3 receptor inverse agonists (Hancock and Fox, 2004). For example, H_3 receptor antagonists/inverse agonists exert proattentional activity in a 5-trial acquisition test performed in spontaneously hypertensive rat pups, often considered as a model for attentional deficits and impulsivity in ADHD patients (Fox *et al.*, 2002). These effects of H_3 antagonists are reversed by H_1 antagonists, which suggests that the latter are attributable to enhanced histamine release. In agreement, H_3 receptor knockout mice display enhanced spatial learning and memory (Rizk *et al.*, 2004).

Histaminergic neurons affect secretion of several pituitary hormones and may also participate in the hormonal responses to stress (Knigge and Warberg, 1991; Schwartz *et al.*, 1991). They are activated during various forms of stress and heavily project to hypothalamic or limbic brain areas (e.g., amygdala or bed nucleus of the stria terminalis) involved in these responses. Furthermore, it seems that the activation of various subpopulations of histaminergic neurons within the tuberomammillary nucleus varies according to the nature of stressful stimuli (Ito, 2000; Miklos and Kovacs, 2003).

A satiating role of endogenous histamine is strongly suggested by several observations, although this view is not in agreement with all experimental data. Weight gain is often experienced by patients receiving first-generation H_1 antihistamines crossing the blood-brain barrier as well as antipsychotics or antidepressants displaying potent H_1 receptor antagonist properties. Central infusion of histamine reduces fat accumulation in leptin-resistant obese mice (Masaki *et al.*, 2001). Histamine neurons projecting to the hypothalamus may be responsible for the food intake suppression induced by the fat cell-produced hormone, leptin. In agreement, intracerebroventricular administration of leptin increases hypothalamic histamine release (Morimoto *et al.*, 2000), whereas histamine depletion by α-fluoromethylhistidine treatment attenuates leptin-induced feeding inhibition (Yoshimatsu *et al.*, 1999). Aged mice with gene disruption of either the H_1 receptor (Masaki *et al.*, 2004) or the histidine decarboxylase gene (Fülop *et al.*, 2003) display adiposity; also both mice display hyperleptinemia, which suggests the existence of a regulatory loop between hypothalamic histamine neurons and leptin-producing cells, the nature of which remains elusive. Following the initial observation that the prototypical H_3 receptor antagonist/inverse agonist thioperamide decreases food intake in rats (Sakata *et al.*, 1990), studies using various compounds belonging to this drug class have confirmed that increased brain histamine release in rodents is associated with anorectic and antiobesity effects (Hancock, 2003). For instance, treatment of mice stabilized on a high-fat diet with A-331440, a potent nonimidazole H_3 receptor antagonist, decreased weight

similarly to dexfenfluramine, reduced body fat, and normalized insulin-resistance test (Hancock *et al.*, 2004). Nevertheless, not all H_3 receptor inverse agonists elicit such effects (Barbier *et al.*, 2004), thioperamide-induced appetite suppression was attributed to taste aversion to this compound (Sindelar *et al.*, 2004); in a mouse model of H_3 receptor disruption, a paradoxical increase in body weight, food intake, and adiposity, together with reductions in energy expenditure, has been reported (Takahashi *et al.*, 2002).

The anticonvulsant properties of endogenous histamine were initially suggested from the occurrence of seizures in epileptic patients, particularly children, following administration of high doses of H_1 receptor antagonists crossing the blood–brain barrier, even those devoid of anticholinergic activity (Sangalli, 1997). The role of histaminergic neurons in preventing seizures, or even the development of epileptogenesis, presumably, in most cases, via H_1 receptor activation, has been shown in several rodent models of epilepsy (Yokoyama, 2001). In agreement, drug-induced changes in histamine synthesis, release, or metabolism confirmed the role of the endogenous amine acting via the H_1 receptor in preventing seizure activity elicited in rodents by pentetrazole, transcranial electrical stimulation, or amygdaloid kindling. Consistently, H_3 antagonists inhibit amygdaloid-kindled seizures, an effect prevented by H_1 antagonists, which suggests the involvement of endogenous histamine (Kamei, 2001). These studies suggest that enhancing brain histamine release via H_3 receptor blockade should represent a novel therapeutic approach for epilepsies.

III. Changes in the Histaminergic System in Schizophrenia

A. Genetic Studies

HMT may be an important target to modulate histaminergic neurotransmission in neuropsychiatric disorders. Among the various HMT polymorphisms that have been identified in the human gene (Aksoy *et al.*, 1996; Chen *et al.*, 2003a; Wang *et al.*, 2002), a common C314T transition located in exon 4 results in decreased levels of enzyme activity (Chen *et al.*, 2003a; Preuss *et al.*, 1998) but was not associated with schizophrenia (Yan *et al.*, 2000).

The human H_1 receptor gene contains an intron in the 5′-flanking untranslated region, close to the translation initiation codon, but the translated region is intronless (De Backer *et al.*, 1998). Several polymorphisms have been identified in the promoter and coding region, but none of them was found to be associated with schizophrenia or response to clozapine (Hong *et al.*, 2002; Mancama *et al.*, 2000, 2002).

The 5′-untranslated region of the human H_2 receptor gene contains an intron but the translated region is intronless. An initial study by Orange *et al.* (1996) has

reported six polymorphisms of the coding region in a UK population. Among them, a A649G transition was found to be associated with schizophrenia. However, none of these variants could be detected in other studies. Several other polymorphisms have been identified in various ethnic groups in the promoter region and one (543G/A) in the coding region of the gene, but none of them was found to be associated with schizophrenia or response to clozapine (Fukushima *et al.*, 2001; Ito *et al.*, 2000; Mancama *et al.*, 2000, 2002).

B. Histamine Neuron Activity

Perfusion of the rat posterior hypothalamus, in which histaminergic cell bodies are located, with dopamine D2 receptor agonists enhances histamine release *in vivo* (Prast *et al.*, 1993). In addition, methamphetamine, a psychotogenic drug which enhances dopamine release in schizophrenic patients (Laruelle *et al.*, 1996), was shown to enhance histamine release in dialysates of rat striatum. This response was completely blocked by haloperidol, an antagonist at dopamine D2-like receptors (Ito *et al.*, 1996). Furthermore, the behavioral sensitization to dopamine agonists, a cardinal feature of schizophrenia, observed following repeated administration of methamphetamine was accompanied by an enhanced basal histamine release in rat striatum. This effect was again blocked by haloperidol, reflecting an increased tonic dopaminergic influence on histaminergic neurons (Ito *et al.*, 1996).

Consistent with these findings on histamine release, methamphetamine also enhances histaminergic neuron activity in mouse brain both acutely and in a long-term fashion. A single administration of methamphetamine markedly increases *tele*-MeHA levels, an index of histamine neuron activity, in the cerebral cortex, striatum, and hypothalamus (Morisset *et al.*, 2002). This enhancing effect of methamphetamine on *tele*-MeHA levels results from the stimulation of histaminergic neurons by endogenous dopamine activating selectively D2 receptors. In agreement, this effect was completely blocked by haloperidol, a D2/D3 receptor antagonist, but remained unchanged either after administration of nafadotride used at a dose inducing a selective blockade of the D3 receptor (Sautel *et al.*, 1995), or in the brain of mice lacking functional D3 receptors. Using [^{125}I]iodosulpride as a ligand, D2-like receptor-binding sites have been evidenced by autoradiography at the level of the tuberomammillary nucleus (Bouthenet *et al.*, 1987), an area in which D3 receptors could not be detected (J. Diaz, personal communication). Therefore, endogenous dopamine may directly activate histamine neurons by interacting with D2 receptors located on their perikarya or dendrites (Morisset *et al.*, 2002), as also supported by retrograde tracing studies combined with immunochemistry showing that dopamine-containing axons emanating from the ventral tegmental area or substantia nigra

project to the tuberomammillary nucleus (Ericson *et al.*, 1989). D2 receptors located on histaminergic nerve endings do not seem to be involved since apomorphine fails to significantly affect histamine release from slices of rat cerebral cortex (Schwartz *et al.*, 1990).

Basal *tele*-MeHA levels were enhanced in various brain regions of sensitized mice showing that repeated administration of methamphetamine induces a long-lasting enhancement of histaminergic neuron activity in the whole brain (Morisset *et al.*, 2002), which is consistent with the increase in histamine release observed in the striatum of sensitized rats (Ito *et al.*, 1996). Like the response to acute administration, this effect of chronic treatment with methamphetamine on *tele*-MeHA levels was blocked by haloperidol, strongly suggesting that it resulted from a sensitized release of dopamine from dopaminergic afferents, leading to a higher degree of activation of D2 receptors present on histaminergic neurons.

In addition to the dopaminergic hypothesis of psychotic disorders, a hypoactivity of glutamatergic transmission has been implicated in schizophrenia. Initially, this glutamatergic hypothesis of schizophrenia was mainly based on the schizophrenia-like psychotomimetic effects of phencyclidine (PCP), which are now mainly attributed to noncompetitive antagonism of the *N*-methyl-D-aspartate (NMDA) receptor (Javitt and Zukin, 1991; Jentsch and Roth, 1999). Consistent with a hyperactivity of histamine neurons in psychotic disorders, it was shown that PCP, in a dose range of 2–10 mg/kg, significantly enhances *tele*-MeHA levels in various mouse brain regions (Itoh *et al.*, 1985, 1986). It was initially suggested that this enhancing effect of PCP on histamine neuron activity involved stimulation of opiate receptors via release of endogenous opioid peptides because it was antagonized by a large dose of naloxone (Itoh *et al.*, 1987) and because direct stimulation of μ-opioid receptors had been shown to increase histamine turnover in the mouse brain (Itoh *et al.*, 1988; Nishibori *et al.*, 1985). However, although it was initially reported to interact with a large number of molecular target sites, PCP was subsequently found to act as a more potent noncompetitive antagonist of the NMDA receptor (Anis *et al.*, 1983) and many data demonstrate that blockade of the NMDA channel is the primary mechanism involved in the effects elicited by PCP (Javitt and Zukin, 1991; Jentsch and Roth, 1999). It could therefore be predicted from the previous studies with PCP that NMDA receptor blockade could activate histamine neurons. Consistent with this proposal, we showed that the effect of PCP is mimicked by MK-801, another NMDA open-channel blocker displaying high potency and selectivity (Wong *et al.*, 1986). MK-801 administration results in an enhancement of *tele*-MeHA levels in the same range as that elicited by PCP and which occurred with a low ED_{50} value (~0.1 mg/kg; Faucard *et al.*, 2006). In addition, a significant increase in *hdc* mRNA expression is induced by PCP administration both in the rostral and caudal parts of the tuberomammillary nucleus (Faucard *et al.*, 2006). Therefore, the strong enhancement of *tele*-MeHA levels and *hdc*-mRNA expression

induced in rodent brain not only by methamphetamine but also by NMDA receptor antagonists further supports the existence of a hyperactivity of histamine neurons in psychotic disorders.

Consistent with this proposal, Prell *et al.* (1995) showed that *tele*-MeHA levels were significantly elevated in the cerebrospinal fluid of patients with chronic schizophrenia, either under neuroleptic treatment or not, indicating that hyperactivity of dopaminergic transmission is associated with an enhanced activity of histaminergic neurons in the disease (Prell *et al.*, 1995). In addition, the decrease in H_1 receptor-mediated responses consistently observed in schizophrenic patients (Nakai *et al.*, 1991; Rauscher *et al.*, 1980) is likely to result from the down-regulation of postsynaptic H_1 receptors induced by the chronic increase in histamine release. In agreement, positron emission tomography (PET) studies using [^{11}C]doxepin revealed a significant decrease in H_1 receptor binding in the frontal and prefrontal cortices and the cingulate gyrus brain of schizophrenic patients (Iwabuchi *et al.*, 2005).

IV. Interactions of Antipsychotic Drugs with the Histaminergic System

A. Interactions of APDs with Histamine Receptors

A large number of antipsychotics are potent H_1 receptor antagonists and block [^{3}H]mepyramine binding to the receptor in rodent and human brain at sub-therapeutic dosages (Quach *et al.*, 1979; Richelson and Souder, 2000). The major part played by the H_1 receptor in the arousal induced by histamine neurons suggests that this blockade of H_1 receptors in brain is involved in the sedative side-effects of many antipsychotic drugs (APDs). In addition, the inhibitory role of endogenous histamine on food intake mediated by the H_1 receptor, namely on the ventromedial nucleus (Sakata *et al.*, 1997), probably accounts for the weight gain that is often experienced by patients receiving antipsychotics displaying potent H_1-receptor antagonist properties. This eventually results in an increased risk of developing a "metabolic syndrome" in patients chronically treated with such agents (Kroeze *et al.*, 2003; Richelson and Souder, 2000). Assuming that blockade of the dopamine D2 receptor is the mechanism of action of APDs, the ratio of K_i's to this receptor and the H_1 receptor of various compounds, which varies largely (Table II), should reflect their ability to block the H_1 receptor at therapeutic dosages and, thereby, exert sedative and pro-obesity side effects. Hence, for instance, olanzapine is one of the most potent H_1 receptor antagonist known so far (Richelson and Souder, 2000) and its marked sedative and weight-gain side-effects are well established. In agreement, sedative APDs at therapeutic dosages were shown to occupy a significant fraction

TABLE II
COMPARED POTENCIES (K_i, nM) OF SEVERAL APDS AT HUMAN HISTAMINE H_1 AND DOPAMINE D2 RECEPTORS

Compounds	Dopamine D2	Histamine H_1	Ratio D2/H_1
Clozapine	210	3.1	68
Haloperidol	2.6	260	0.01
Olanzapine	20	0.087	230
Quetiapine	770	19	40
Risperidone	3.8	5.2	0.7
Sertindole	2.7	320	0.01
Ziprasidone	2.6	4.6	0.6

Values are derived from Richelson and Souder (2000).

of cerebral H_1 receptors in living rodents (Quach *et al.*, 1979) and weight-gain propensity of several APDs in patients were significantly correlated to their relative potencies at these receptors (Kroeze *et al.*, 2003).

An intriguing observation is that several APDs and a number of tricyclic antidepressants are very potent and competitive inhibitors of the H_2 receptor-linked adenylyl cyclase on brain membranes (Green and Maayani, 1977; Green *et al.*, 1977; Kanof and Greengard, 1978). This has led to the suggestion that blockade of the H_2 receptor might account at least partially for the clinical activity. However, for unclear reasons, such a potent blockade could not be observed on intact cell preparations (Dam Trung Tuong *et al.*, 1980).

Whereas typical APDs were ineffective at the H_3 receptor, the atypical APD clozapine has been shown to block the rodent H_3 receptor as evidenced on the release of noradrenaline or serotonin from brain slices and in radioligand binding assays to the recombinant or native receptor (Alves-Rodrigues *et al.*, 1996; Kathmann *et al.*, 1994; Lovenberg *et al.*, 2000; Morisset *et al.*, 1999). This led to the speculation that some of its "atypical" properties might be due to H_3 receptor antagonism. However, consistent with a species-related heterogeneity of H_3 receptors (Schwartz *et al.*, 2001), clozapine does not significantly interact with the recombinant human H_3 receptor (Lovenberg *et al.*, 2000).

Clozapine displays a submicromolar affinity at the human H_4R (de Esch *et al.*, 2005), but plasma and brain concentrations associated with clinical responses meet or exceed these values. Even more interesting is that clozapine, which acts as an antagonist at various receptors, behaves as a full agonist at the recombinant human H_4R and at the H_4R present on human eosinophils (Buckland *et al.*, 2003; Liu *et al.*, 2001a,b; Oda *et al.*, 2000). Because the H_4R is mainly expressed on hematopoietic cells, one might therefore speculate that agranulocytosis, which often limits clozapine effectiveness, is related to H_4R activation.

B. Modulation of Histamine Neuron Activity by APDs

Consistent with a tonic stimulation of histamine neurons by endogenous dopamine interacting with D2 receptors present on histaminergic cell bodies, typical APDs, for example, haloperidol, decrease histamine neuron activity (Morisset *et al.*, 1999).

In contrast, atypical neuroleptics, for example, clozapine, enhance histamine turnover, an effect related to 5-HT_2 receptor blockade. Ketanserin, a preferential 5-HT_{2A} receptor antagonist, mimicked the enhancing effect of atypical antipsychotics on *tele*-MeHA levels in mouse cerebral cortex, striatum, and hypothalamus. DOI, a 5-$HT_{2A/2C}$ receptor agonist, did not modify *tele*-MeHA level but strongly reversed the effect of clozapine (Morisset *et al.*, 1999). These findings therefore show that endogenous serotonin tonically inhibits HA neurons via 5-HT_{2A} receptors, an effect blocked by clozapine. These 5-HT_{2A} receptors could be located on HA neurons themselves, on interneurons, or nearby axon terminals impinging on the formers. In agreement with the blockade of 5-HT_{2A} receptors by atypical neuroleptics, the effect of clozapine was not additive with that of ketanserin. This strongly suggests that the activation of histaminergic neurons by clozapine (and other novel antipsychotics) is entirely attributable to 5-HT_{2A} receptor blockade (Morisset *et al.*, 1999).

A recognized advantage of atypical APDs compared to typical APDs is their arousing and procognitive effects resulting in a significant efficacy against negative symptomatology. The positive functional role attributed to histaminergic neurons in wakefulness, attention, and cognition suggests that this property of atypical antipsychotics could be related at least partially to their unique ability to activate histamine neurons.

V. Role of Histaminergic Neurons in Schizophrenia

Overdose of a variety of classical H_1-antagonists was repeatedly reported to result in toxic psychoses with hallucinations resembling schizophrenia and the hallucinogenic potential of these drugs has even led to abuse (Sangalli, 1997). The increase in dopamine release and the blockade of dopamine uptake induced by such compounds in the striatum, rather than blockade of H_1 receptors, presumably explain their abuse potential (Dringenberg *et al.*, 1998). In agreement, dopamine turnover remains unchanged in the forebrain from H_1 receptor knockout mice (Yanai *et al.*, 1998).

In several open-label clinical trials, famotidine, an H2R antagonist with limited brain penetration, was found to display an antipsychotic efficacy (Kaminsky

et al., 1990; Oyewumi *et al.*, 1994; Rosse *et al.*, 1996), a finding which remains to be confirmed in controlled studies.

In the search of new antipsychotic agents, increasing evidence supports the therapeutic potential of H_3 receptor antagonists/inverse agonists for the symptomatic treatment of schizophrenia. The latters do not change spontaneous locomotor activity when they are used alone (Clapham and Kilpatrick, 1994; Imaizumi and Onodera, 1993; Pillot *et al.*, 2002b) and do not induce locomotor sensitization (Komater *et al.*, 2003). However, the locomotor activation elicited in rat and mouse by various dopaminergic agonists such as amphetamine, methamphetamine, and apomorphine is attenuated by thioperamide and ciproxifan, two standard H_3 receptor antagonists/inverse agonists (Clapham and Kilpatrick, 1994; Morisset *et al.*, 2002). Also ciproxifan, a potent H_3 receptor antagonist, significantly decreases the stereotypies induced in mice by methamphetamine and apomorphine (Sadakhom, C.; Frances, H., and Arrang, J. M. in preparation). Consistent with these findings, the effect of methamphetamine on locomotor activity and stereotypic behavior was less pronounced in H_3 receptor knockout mice (Toyota *et al.*, 2002).

In another animal model of psychosis (Andine *et al.*, 1999; Carlsson and Carlsson, 1990), the locomotor hyperactivity induced in rodents by the NMDA receptor antagonist MK-801 is also markedly attenuated by H_3 receptor antagonists/inverse agonists (Faucard *et al.*, 2006).

In DBA/2 mice in which sensorimotor-gating deficits, which are considered as cardinal signs of the disease, occur naturally, H_3 receptor antagonists/inverse agonists also improve gating as shown by the increase that they induce in prepulse inhibition of startle and N40 auditory-evoked response (Browman *et al.*, 2004; Fox *et al.*, 2005).

The neurochemical mechanisms underlying these antipsychotic-like effects induced by H_3 receptor blockade remain unknown. Facilitation of histamine release via H_3 autoreceptor blockade may be involved. However, a histamine neuron hyperactivity being already observed in schizophrenia, this would imply that histaminergic neurons are not directly involved in psychotic symptoms but are involved in a compensatory manner. The further enhancement of histamine neuron activity induced by H_3 receptor antagonists/inverse agonists would therefore attenuate psychotic symptoms. Consistent with such a hypothesis, the time course of hyperlocomotion and activation of histamine neurons induced by methamphetamine do not parallel (Morisset *et al.*, 2002), and enhancement of histamine release induced by histidine loads or inhibitors of histamine catabolism have also been reported to reduce methamphetamine-induced locomotor activity (Itoh *et al.*, 1984; Ito *et al.*, 1997).

Alternatively, a direct involvement of histamine in schizophrenic symptoms cannot be ruled out. H_3 receptors are present at high densities on many perikarya

and/or dendrites of intrinsic neurons in the cerebral cortex, basal ganglia, and limbic areas (Pillot *et al.*, 2002a,b). Therefore, the hyperactivity of histamine neurons reported in schizophrenia and in animal models of the disease might enhance the activation of these postsynaptic H_3 receptors. Their blockade would lead to antipsychotic properties of H_3 receptor antagonists.

Whatever the mechanisms involved, recent data show the existence of strong but complex functional interactions between endogenous histamine and dopamine in the brain. Ciproxifan used alone has no effect but strongly modulates the effects of methamphetamine on neuropeptide mRNA expression not only in the caudate-putamen but also in the nucleus accumbens (Pillot *et al.*, 2003). The synergism between the two drugs on enkephalin neurons and their antagonism on substance P/dynorphin neurons may suggest direct interactions between H_3 receptors and dopamine receptors. H_3 receptor activation inhibits dopamine D_1 receptor-mediated cAMP formation in the rat striatum (Sanchez-Lemus and Arias-Montano, 2004) and the expression of H_3 receptors is influenced by endogenous activation of D_1 receptors (Ryu *et al.*, 1996). Presynaptic H_3 heteroreceptors inhibit dopamine synthesis (Molina-Hernandez *et al.*, 2000) and release (Schlicker *et al.*, 1993). Although they do not appear to regulate dopamine neuron activity *in vivo* under basal conditions (Imaizumi and Onodera, 1993; Miyazaki *et al.*, 1997; Oishi *et al.*, 1990b), the inhibition of dopamine neuron activity by H_3 heteroreceptors may become operating in schizophrenia because of an enhanced histamine release, as shown by the potentiation of methamphetamine-induced accumbal dopamine release induced by H_3 receptor antagonists/inverse agonists (Munzar *et al.*, 2004). In addition, in freely moving rat microdialysis studies, H_3 receptor antagonists do not enhance dopamine release in striatum but enhance it in frontal cortex (Fox *et al.*, 2005).

Both pharmacological interactions between H_3 and D2 receptors and pharmacokinetic drug-drug interactions may account for the complex interactions reported between H_3 receptor antagonists/inverse agonists and neuroleptics. In one study, the imidazole derivative ciproxifan potentiated the enkephalin, neurotensin, and c-fos expression induced in rat caudate-putamen and nucleus accumbens (Pillot *et al.*, 2002b). By contrast, thioperamide, another imidazole compound, decreased haloperidol-induced c-fos expression in the rat dorsolateral striatum but not in the nucleus accumbens (Hussain *et al.*, 2002). Similar discrepancies were also found in behavioral studies. Ciproxifan and thioperamide potentiated haloperidol-induced catalepsy in the rat (Pillot *et al.*, 2002b; Zhang *et al.*, 2005) but not in the mouse (Morisset *et al.*, 1999). In the rat, the potentiation of catalepsy was likely to result at least partially from an inhibition of cytochrome P450 enzymes by imidazole derivatives (Yang *et al.*, 2002), and two nonimidazole H_3 receptor antagonists/inverse agonists tended to attenuate risperidone-induced catalepsy (Zhang *et al.*, 2005).

VI. Conclusions

The present chapter testifies how our knowledge of the molecular neurobiology of cerebral histaminergic systems and their implications in physiological functions, for example, arousal, cognition, or control of food intake, has progressed during the last years. This appears as the result of the development of reliable research tools such as selective ligands for the various receptor subtypes or genetically modified mice. Recent findings support the possible implication of the histaminergic system in schizophrenia and therapeutic utility and/or side effects of APDs. H_3 receptor antagonists/inverse agonists raise a great interest as innovative therapeutics in various CNS disorders including schizophrenia and are currently undergoing clinical trials. The results of these clinical studies are now awaited to confirm this potential interest and they should teach us a lot about the role of the histaminergic system in the human brain.

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CANNABINOIDS AND PSYCHOSIS

Deepak Cyril D'Souza

Department of Psychiatry, Yale University School of Medicine
VA Connecticut Healthcare System
West-Haven, Connecticut 06516, USA

Recent epidemiological studies and advances in understanding of brain cannabinoid function have renewed interest in the long-recognized association between cannabinoids and psychosis. This chapter presents evidence supporting and refuting the association between cannabinoids and psychosis. Cannabinoids can induce acute transient psychotic symptoms or an acute psychosis in some individuals. What makes some individuals vulnerable to cannabinoid-related psychosis is unclear. Also clear is that cannabinoids can also exacerbate psychosis in individuals with an established psychotic disorder, and these exacerbations may last beyond the period of intoxication. Less clear is whether cannabis *causes* a persistent *de novo* psychosis. The available evidence meets many but not all the criteria for causality, including dose–response, temporality, direction, specificity, and biological plausibility. On the other hand, the large majority of individuals exposed to cannabinoids do not experience psychosis or develop schizophrenia and the rates of schizophrenia have not increased commensurate with the increase in rates of cannabis use. Similar to smoking and lung cancer, it is more likely that cannabis exposure is a component cause that interacts with other factors, for example, genetic risk, to "cause" schizophrenia. Nevertheless, in the absence of known causes of schizophrenia, the role of component causes such as cannabis

INTERNATIONAL REVIEW OF NEUROBIOLOGY, VOL. 78
DOI: 10.1016/S0074-7742(06)78010-2

0074-7742/07 $35.00

exposure (exogenous hypothesis) is important and warrants further study. There is also tantalizing evidence from postmortem, neurochemical, and genetic studies suggesting CB1 receptor dysfunction (endogenous hypothesis) in schizophrenia that warrants further investigation. Further work is necessary to identify those factors that place individuals at higher risk for cannabinoid-related psychosis, to identify the biological mechanisms underlying the risks and to further study whether CB1 receptor dysfunction contributes to the pathophysiology of psychotic disorders.

I. Introduction

> . . . acute psychotic reactions, generally lasting but a few hours, but occasionally as long as a week; the reaction seemed dose related and its main features included paranoid ideation, illusions, hallucinations, delusions, depersonalization, confusion, restlessness and excitement. There can be delirium, disorientation and marked clouding of consciousness . . .
>
> In 'Du Haschisch et d l'alientation mentale' J. J. Moreau de Tours (1845) (Moreau, 1973).

An association between cannabis and psychosis has long been recognized. However, recent advances in the understanding of cannabinoid receptor function have renewed in the association between cannabis and psychosis. In addition to epidemiological studies, there are case reports of psychosis following cannabis use, reports of psychosis in surveys of cannabis users from community samples, and pharmacological studies with various cannabinoid compounds. These data are relevant to an exogenous hypothesis according to which the consumption of cannabinoid compounds is associated with psychotic disorders. We also suggest an endogenous hypothesis according to which brain cannabinoid receptor (CB1) dysfunction may contribute to the pathophysiology of psychosis and/or schizophrenia, and further, that the putative CB1 receptor dysfunction maybe unrelated to the consumption of cannabinoid compounds. These two hypotheses are by no means mutually exclusive, and may in fact interact.

The data supporting an association between cannabis consumption and the manifestation of psychotic symptoms in humans is now reviewed. However, a review of the literature will not be complete without a discussion about the constituents of cannabis. The principal active ingredient of cannabis is delta-9-tetrahydrocannabinol (Δ-9-THC). However, in addition to Δ-9-THC, herbal cannabis contains nearly 70 cannabinoid compounds, including cannabidiol (CBD), Δ-8-tetrahydrocannabinol, cannabinol, cannabigerol, and also terpenoids and flavonoids (Elsohly and Slade, 2005). These constituent compounds may modulate the effects of Δ-9-THC and may also have "entourage" effects (Mechoulam and Ben-Shabat, 1999; Russo and McPartland, 2003). The principal active metabolite of Δ-9-THC, 11-hydroxy-THC is more potent than Δ-9-THC. The time course of 11-hydroxy-THC blood levels correlates well with the psychological effects of

inhaled and oral Δ-9-HC reviewed in Agurell *et al.* (1986). CBD may offset some Δ-9-THC effects by its anxiolytic effects (Guimaraes *et al.*, 1994; Zuardi *et al.*, 1982), antipsychotic-like effects (Zuardi *et al.*, 1991, 1995, 2006), and may block the conversion of Δ-9-THC to the more psychoactive 11-hydroxy-THC (Bornheim *et al.*, 1995). Therefore, the net effect of herbal cannabis is a composite of its constituents. The CBD content of cannabis varies greatly and some samples of cannabis have been reported to be devoid of CBD (Pitts *et al.*, 1990, 1992). Thus, a relatively lower CBD content of cannabis has been implicated in the occurrence of psychotic and anxiety reactions with cannabis use (Solomons and Neppe, 1989; Solomons *et al.*, 1990). An example is South African cannabis, also known as dagga, which has very low levels of CBD compared to other varieties of cannabis obtained elsewhere. Naturalistic studies suggest an association between dagga consumption and high rates of psychotic symptom (Solomons and Neppe, 1989; Solomons *et al.*, 1990).

There are some data from studies with synthetic cannabinoids including dronabinol, nabilone, and levonantradol that are informative about the psychotic adverse effects of cannabinoids (Fig. 1). Dronabinol is synthetic Δ-9-THC. The 9-*trans*-ketocannabinoid Nabilone (Cesamet®) is a synthetic analogue of Δ-9-THC that was developed as an antiemetic and is available in Europe and Canada. Levonantradol, also a synthetic cannabinoid, was developed as an analgesic agent but abandoned because of a high incidence of intolerable behavioral side effects.

Evidence for an association between cannabis and psychosis comes from several sources, including case series of psychosis following cannabis use, autobiographical accounts, and surveys of cannabis users in the general population, epidemiological studies, and pharmacological studies with various cannabinoid compounds.

II. Ancedotal Reports

A. Autobiographical Accounts

There are several exquisitely detailed autobiographical accounts of the effects of cannabinoids. In perhaps one of the first detailed accounts of cannabis effects, Moreau de Tours (1845) described acute, transient, dose-related psychotic reactions lasting hours to days following hashish use (Moreau, 1973). The reaction was characterized by paranoid ideation, illusions, hallucinations, delusions, depersonalization, confusion, restlessness, and excitement. Among other effects of cannabis, Marshall described grandiosity ("my powers became superhuman, my knowledge of the universe was infinite, and so on") (Marshall, 1897). At higher doses he described disturbing hallucinations ("demons"). While informative, these individual accounts have limited generalizability.

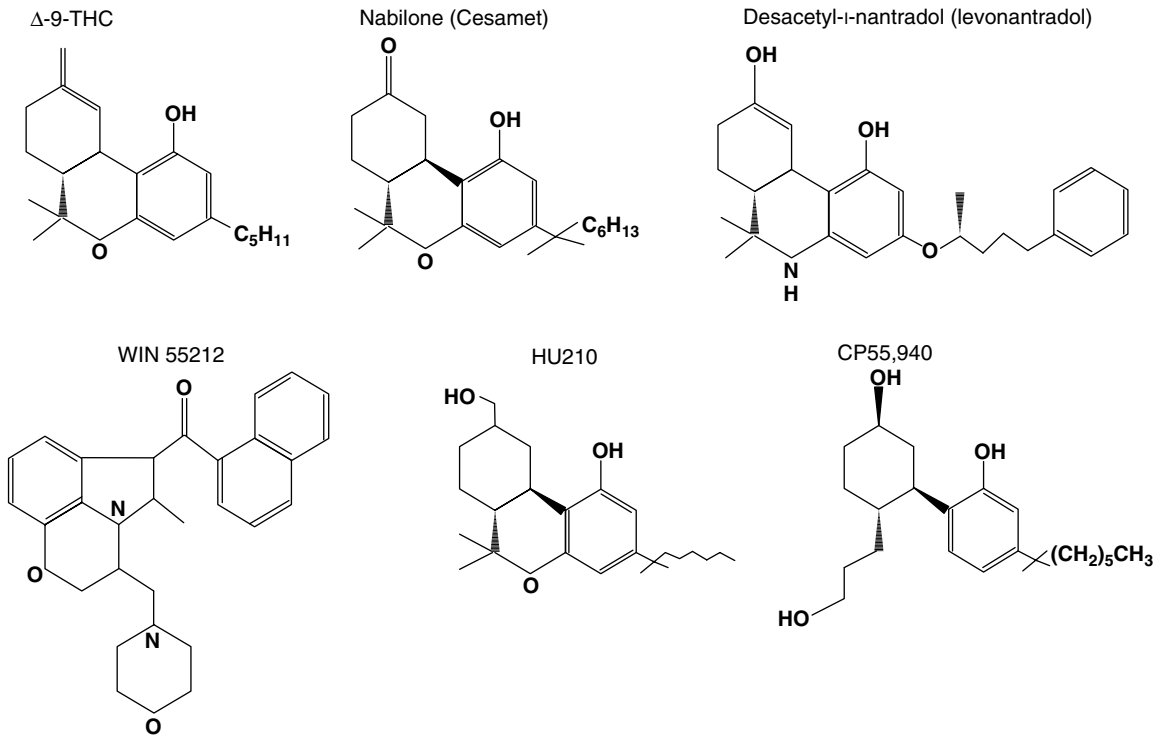

FIG. 1. Natural and synthetic cannabinoid receptor agonists.

B. Surveys of Cannabis Users

Some of the limitations of individual accounts can be addressed in surveys of large groups of cannabis users from community samples. Thomas surveyed 1000 New Zealanders aged 18–35 years about the effects of cannabis (Thomas, 1996). Thirty-eight percent of respondents admitted to cannabis use. The most common adverse effects included anxiety and panic attacks (22%) followed by psychotic symptoms such as auditory hallucinations and persecutory ideas (15%). A significant relationship between panic attacks and psychosis was found. However, the survey, failed to find a dose–response relationship between the levels of cannabis use and the occurrence of these symptoms. As discussed later, there are challenges to accurately estimate dose–response relationships from naturalistic data. In a study of young Australian cannabis users ($n = 268$), about 21% reported negative effects that included paranoia (Reilly *et al.*, 1998). Green *et al.* (2003) reviewed pooled data ($n > 2500$) from surveys of cannabis users that used closed questions and found that 51.4% of the sample reported paranoia whereas 19.8% reported hallucinations while under the influence of cannabis. Thus, psychotic symptoms are not uncommonly experienced under the influence of cannabis. However, survey data too have their limitations, including sampling bias, reliance on self-report, lack of structured scales to assess psychosis, lack of reliable dose–response data, and so on.

At this point, it would be important to make the distinction between a psychotic disorder and psychotic symptoms, since these terms are somewhat poorly defined in the literature. Psychotic disorder refers to a condition characterized by *persistent* psychotic symptoms accompanied by functional deficits. Further, most discussions associating cannabis and psychosis have referred mainly to positive symptoms (hallucinations, perceptual alterations, delusions, paranoia, ideas of reference, disorganized speech, and disorganized behavior). However, any discussion of schizophrenia would be incomplete without reference to negative symptoms (emotional withdrawal, blunted affect, amotivation, alogia, and social withdrawal) and cognitive deficits (deficits in memory, attention, and executive function). Furthermore, the inclusion of schizophrenia-spectrum conditions such as schizotypy makes for some interesting findings. Several groups have found higher scores on measures of schizotypy, positive psychotic symptoms, and perceptual alterations in cannabis users (Dumas *et al.*, 2002; Nunn *et al.*, 2001; Skosnik *et al.*, 2001). Verdoux *et al.* (2003c) studied the association between cannabis use and psychotic dimensions in a nonclinical sample (571 college students) 18–51 years of age and found an association between cannabis use and positive and negative dimensions of psychosis and a correlation between the frequency of use and intensity of symptoms. This relationship appeared to be specific for cannabis use as alcohol use was not associated with dimensions of psychosis. Further the association with cannabis use was specific to positive and negative symptoms of psychosis but not depression.

Another limitation of cross-sectional surveys is that they are not very informative on the direction of causality. Thus, cannabis use may be a consequence rather than a cause of psychosis or cannabis use and psychosis may be independently associated with some common risk factor(s). Prospective studies have addressed some of these limitations. Verdoux *et al.* (2003a) investigated the impact of cannabis use on the onset of psychotic experiences using an experience sampling method, a self-reported structured diary technique that has been validated as a method of collecting information on psychotic experiences in daily life. Subjects were instructed to respond to randomly programmed cues from a wristwatch to describe their present substance use and psychotic experiences five times a day over 7 consecutive days. There was a clear temporal relationship between cannabis use and the acute occurrence of psychotic-like abnormal perceptions.

C. Psychosis in Cannabis Users from Community Samples

Using data from the US Epidemiological Catchment Area (ECA) study, Tien and Anthony (1990) found that daily cannabis use doubled the risk of reporting psychotic symptoms after adjusting for baseline alcohol use and psychiatric diagnosis. Similarly, in the Australian National Survey of Mental Health and Well-Being, 17.2% of individuals diagnosed with cannabis dependence screened positive for a psychotic disorder (Degenhardt *et al.*, 2001).

D. Naturalistic Case Series

In a review, Hall and Degenhardt (2004) found 397 cases of "cannabis psychosis" reported in the literature. In perhaps the earliest case series, Chopra and Smith (1974) described 200 patients admitted to a psychiatric hospital in India for psychosis following cannabis use (Chopra, 1973; Chopra and Smith, 1974). The psychosis was typically preceded by ingestion of large doses of cannabis and was characterized by hallucinations, paranoia, delusions, depersonalization, emotional lability, amnesia, confusion, and disorientation. One-third of the patients reportedly had no previous psychiatric history, suggesting that the heavy cannabis abuse in these subjects was not a sign of preexisting psychopathology. Further, the use of more potent forms of cannabis, for example, hashish, was associated with a quicker onset of psychosis suggesting a dose–response. Similar case series have been reported from other geographical areas, including Denmark (Arendt *et al.*, 2005), Sweden (Bernhardson and Gunne, 1972), the Carribean (Harding and Knight, 1973), the United Kingdom (Carney *et al.*, 1984; Mathers *et al.*, 1991), the United States (Talbott and Teague, 1969b; Tennant and Groesbeck, 1972a), Scotland (Wylie *et al.*, 1995), and South Africa (Rottanburg *et al.*, 1982).

Most of the these studies suggest that psychotic episodes resolved completely when cannabis use stopped but recurred with the resumption of cannabis use reviewed in Hall and Solowij (1998) and typically resolved fairly quickly in comparison with endogenous psychoses (Basu *et al.*, 1999; Carney *et al.*, 1984; Chaudry *et al.*, 1991; Cohen, 1994; Kolansky and Moore, 1971a; Rottanburg *et al.*, 1982; Talbott and Teague, 1969a; Thacore, 1973; Thacore and Shukla, 1976; Wylie *et al.*, 1995). Further, some studies suggested that patients with preexisting mental problems had a less favorable outcome (Bernhardson and Gunne, 1972; Bromberg, 1939; Chopra and Smith, 1974; Palsson *et al.*, 1982; Tennant and Groesbeck, 1972b).

In most of the studies discussed thus far, cases/patients were followed no greater than 3 months after remission of psychosis and hence, long-term outcome of these cases could not be conclusively determined. Arendt *et al.* (2005) reported the long-term outcome of a cohort of patients treated for cannabis-induced psychotic disorder extracted from the Danish Psychiatric Central Register. All patients treated for ICD-10 cannabis-induced psychotic disorder between 1994 and 1999 who had never been previously treated for psychosis ($n = 535$) *were* examined for the development of schizophrenia-spectrum disorder in the ensuing years (Table I). Schizophrenia-spectrum disorders were diagnosed in 44.5% of the sample. When the diagnosis was broadened to include new psychotic episodes of *any* type, the diagnosed sample increased to 77.2%. Further, only 15.9% of individuals were not in psychiatric care at the time of long-term follow-up. About half of the patients received the diagnosis of schizophrenia-spectrum disorder more than a year after seeking treatment for a cannabis-induced psychosis. The patients had an earlier age of onset of schizophrenia compared to a control group without a history of cannabis-induced psychosis. This study is the first to show that such psychotic symptoms induced by cannabis may be the first manifestations of a long-term psychotic disorder such as a schizophrenia-spectrum disorder.

These case series have some shortcomings. First, it is possible that the individuals who developed psychosis after using cannabis were not "healthy" and carried some risk for developing a psychotic disorder. Here, it is important to point out that other than family history there are no other known risks factors that are either specific and/or sensitive predictors of the future development of a psychotic disorder. Further, even though having a first-degree relative diagnosed with schizophrenia increases the risk of developing schizophrenia by 9–18 times, more than 80% of individuals diagnosed with schizophrenia do not have an affected first-degree relative and over 60% do not have an affected first- *or* second-degree relative (Gottesman and Shields, 1982). As discussed later, genetic factors may influence the risk of psychosis associated with cannabis exposure (Caspi *et al.*, 2005). Second, since individuals who use cannabis often co-use other drugs, it is unclear whether the use of other drugs contributed to the development

TABLE I
RISK OF MENTAL ILLNESS FOLLOWING HOSPITALIZATION FOR CANNABIS-INDUCED PSYCHOSIS PATIENTS TREATED FOR MENTAL OR BEHAVIORAL DISORDERS AFTER INDEX POINT ($N = 535$)

Diagnosis[a]	Within 3 years *n* (%)	After 3 years *n* (%)	Total follow-up *n* (%)
Schizophrenia-spectrum disorder[b]	197 (36.8)	41 (7.7)	238 (44.5)
Persistent delusional disorder (F22)	18 (3.4)	4 (0.7)	22 (4.1)
Other or non-organic psychotic disorder (F28/F29)	5 (0.9)	3 (0.6)	8 (1.5)
Bipolar affective disorder	12 (2.2)	6 (1.1)	18 (3.4)
Acute and transient psychotic disorder	28 (5.2)	7 (1.3)	35 (6.5)
Cannabis-induced psychosis	78 (14.6)	1 (0.2)	79 (14.8)
Other drug-induced psychosis	11 (2.1)	2 (0.4)	13 (2.4)
Depression, anxiety or personality disorder	29 (5.4)	8 (1.5)	37 (6.9)
No treatment			85 (15.9)
Total			535 (100)

[a]Patients are entered only once, in a hierarchical manner as described in the method section.
[b]Schizophrenia (ICD–10 code F20), schizotypal disorder (F21) or schizoaffective disorder (F25).

of psychosis. Third, only a minority of case series studies employed standardized measures of psychosis. Fourth, the amount of cannabis use preceding the psychotic episode was not quantified limiting any speculation about dose–response relationships. In contrast, the temporal relationship between cannabis use and psychosis, the fact that psychosis resolved with abstinence from the drug and recurred with renewed use, lends support to the notion that the relationship between cannabis exposure and the development of psychosis is not merely coincidental.

On the basis of some similarities between the phenomenology of psychosis associated with cannabis use and the psychosis of schizophrenia, some (Chaudry *et al.*, 1991; Ghodse, 1986; Mathers *et al.*, 1991; Rolfe *et al.*, 1993; Rottanburg *et al.*, 1982; Thacore, 1973; Thacore and Shukla, 1976) but not others (Hall and Degenhardt, 2004; Imade and Ebie, 1991; McGuire *et al.*, 1994, 1995; Thomas, 1993; Thornicroft, 1990) have argued for the inclusion of "cannabis psychosis" as a distinct nosological entity. Arguments challenging the validity of "cannabis psychosis" as a distinct diagnostic entity should not be confused with the debate on the association between cannabis and psychosis.

As mentioned earlier, it is difficult to derive dose–response relationships from naturalistic data for a number of reasons. The reliability of self-reported, long-term, retrospective estimates of cannabis use is unclear. Individuals who use cannabis will often share a "joint" with one or more individuals, thus estimating

the dose consumed by the individual may be difficult. Cannabis is consumed in many different ways, for example, "joints," "bongs," and so on, which are not equivalent. The estimates of the number of lifetime exposures cannot accurately reflect the actual dose of Δ-9-THC that is consumed. Finally, the Δ-9-THC content of cannabis varies greatly. The Potency Monitoring program, a collaboration between the University of Mississippi and the National Institute on Drug Abuse (NIDA), provides analytical data about the potency of confiscated marijuana seized in the United States. In the most recent report covering the last 10 years on ∼30,000 cannabis samples, 207 hashish samples, and 86 hash oil samples there appears to be an upward trend in the average THC content of confiscated cannabis (Mehmedic *et al.*, 2005). The Δ-9-THC content of cannabis doubled from 3.48% in 1994 to 7.08% in 2004. While there was no consistent increase in Δ-9-THC content in hashish samples from 1994 to 1999, the average potency of hashish samples increased from 4.16% Δ-9-THC in 2000 to 11.2% in 2004. No potency trends were observed for hash oil samples. Finally, there was no change in the average levels of the other cannabinoids (CBD, CBC, CBG, and CBN) in the cannabis samples over the reported time frame. Similarly, the average Δ-9-THC content of Dutch cannabis, Dutch hashish, and imported hashish has significantly increased between 1999 and 2005 (Niesink *et al.*, 2005). For example, in 2005, the average Δ-9-THC content of Dutch home-grown cannabis (Nederwiet) was 17.73%, and was nearly three times higher than that of imported cannabis (6.7% Δ-9-THC). Dutch hashish (Nederhasj) contained 26% Δ-9-THC in 2005, compared with 16.9% THC in imported hashish. In summary, deriving accurate dose–response from naturalistic data may have significant limitations.

III. Epidemiological Studies

Epidemiological studies have provided *the* major contribution to the evidence supporting an association between cannabis and psychosis (Table II). The study that first brought significant attention to the topic was a large historical, longitudinal cohort study of all Swedes conscripted between 1969 and 1970 (Andreasson *et al.*, 1987). Since Sweden mandates military service, 97% of males aged 18–20 years were included. The relationship between self-reported cannabis use at the time of conscription and psychiatric hospitalization for schizophrenia in the ensuing 15 years was examined. A dose–response relationship was observed between cannabis use at conscription (age 18 years) and schizophrenia diagnosis in the following 15 years. Individuals who reported having used cannabis more than 50 times were 6 times more likely than nonusers to have been diagnosed with schizophrenia 15 years later. Adjusting for other relevant risk factors including

TABLE II
EPIDEMIOLOGICAL STUDIES

References	*N*	Design	Instrument	Age range at f/u (years)	Outcome		Adjusted Risk
Arendt *et al.* 2005	535	Longitudinal follow up of cannabis-induced psychosis (Denmark)	Registry	Not specified (estimated as 32 years)	New psychotic episode of any type diagnosed in 77.2%. Schizophrenia-spectrum disorders diagnosed in 44.5% Earlier onset of schizophrenia		–
Ferdinand *et al.* 2005	1580	Prospective cohort (The Netherlands)	CIDI CBCL	18–30	Psychotic symptoms	Lifetime CB use	2.8
Henquet *et al.* 2005	2437	Prospective cohort (Germany)	M-CIDI	18.3–21.8	Any psychotic symptom	Lifetime CB use	1.7
Stefanis *et al.* 2004	3500	Cross-sectional cohort of adolescents (Greece)	CAPE PDI	18	Positive and negative symptoms	Lifetime CB use until age 19 years	4.3
Fergusson, 2003	1011	Birth cohort (Christchurch)	SCL90	21	Psychotic symptoms	DSM-IV CB dependence at 21 years	1.8
Arseneault *et al.* 2002	759	Longitudinal, prospective, birth cohort (Dunedin)	DSM-IV	26	Schizophrenia and schizophreniform disorders	Lifetime CB use until age 15 and 18 years	3.12
Weiser *et al.* 2002	50,413	Longitudinal conscript cohort (Israel)	registry	4–15	Hospitalization for schizophrenia	Lifetime drug use at 16–17 years	2
van Os *et al.* 2002	4095	Longitudinal population-based 3 year follow-up (The Netherlands)	CIDI, SCID	18–64	Any psychotic symptoms	Lifetime CB use at 16–17 years	2.76
Zammit *et al.* 2002	50,053	Longitudinal conscript cohort (Sweden)	Registry	45–47	Hospitalization for schizophrenia	>50 exposures to CB at age 18 years	3.1
Andreasson *et al.* 1987	45,570	Longitudinal conscript cohort (Sweden)	Registry	33–35	Hospitalization for schizophrenia	>50 exposures to CB at age 18 years	2.3

psychiatric diagnosis other than psychosis at conscription reduced but did not eliminate the higher risk (odds ratio = 2.3) of schizophrenia conferred by cannabis use.

A reanalysis and extension of the same Swedish conscript cohort reconfirmed that heavy cannabis users by the age of 18 years were 6.7 times more likely than nonusers to be hospitalized for schizophrenia in the following 27 years (Zammit *et al.*, 2002). This study addressed the confounding effects of concomitant use of other drugs of abuse, premorbid personality traits, and cannabis use as a form of self-medication of schizophrenia. The adjusted odds ratio for cannabis use and schizophrenia remained significant (1.2), despite adjusting for a number of confounds, including low IQ, urbanicity, cigarette smoking, poor social integration, function, and stimulant use. Further, after controlling for the possibility that cannabis use is a consequence of prodromal manifestations of psychosis by excluding subjects who developed schizophrenia within 5 years of conscription, the finding of an increased risk of schizophrenia conferred by cannabis use persisted. The authors concluded that cannabis use was associated in a causal way with an increased risk of developing schizophrenia and that 13% of cases of schizophrenia would be averted if cannabis use were prevented.

The historical studies have been complemented by a number of recent prospective cohort studies. In a general population birth cohort study of 1037 people born in Dunedin, New Zealand, and followed through until age 26 years, cannabis use conferred a higher risk for the subsequent development of schizophrenia (Arseneault *et al.*, 2002). One of the strengths of this study was that it collected data on self-reported psychotic symptoms at age 11 years, that is, to address whether psychosis preceded cannabis use. Self-reported cannabis at both ages 15 and 18 years use was also collected. Further, the entire sample was assessed at age 26 years using a standardized psychiatric interview that allowed the determination of both schizophrenia symptoms and disorder. Compared to nonusers, individuals using cannabis at ages 15 and 18 years had higher rates of psychotic symptoms and schizophreniform disorder at age 26 years, even after controlling for psychotic symptoms pre-dating the onset of cannabis use. Cannabis users at age 15 years had a higher rate (OR = 3.1) of developing schizophreniform disorder at age 26 years, even after controlling for psychotic symptoms pre-dating the onset of cannabis use (Fig. 2).

In The Netherlands Mental Health Survey and Incidence Study (NEMESIS), 4045 psychosis-free individuals and 59 individuals with a psychotic disorder were assessed at baseline, 1 and 3 years (van Os *et al.*, 2002) using a measure of psychosis. Individuals using cannabis at baseline were nearly three times more likely to manifest psychotic symptoms at follow-up even after adjustment for a range of factors. Further, a dose–response relationship was established with the highest risk (odds ratio = 6.8) for the highest level of cannabis use. The relationship between cannabis use and psychotic symptoms was stronger for cases with

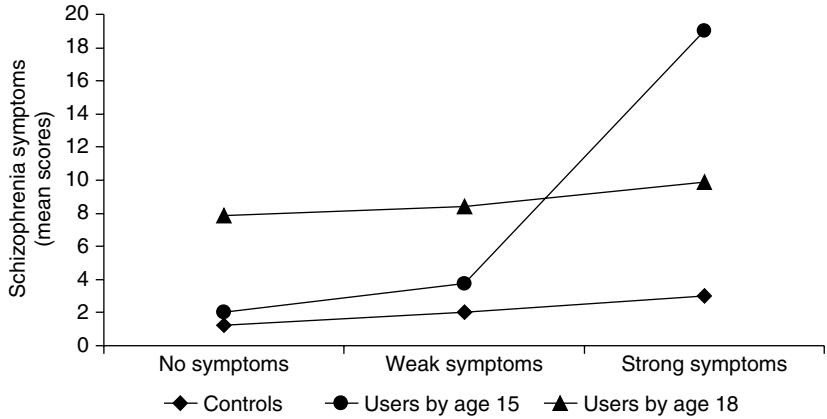

FIG. 2. Interaction between cannabis use at age 18 and psychotic symptoms at age 11 in predicting schizophrenia symptoms.

more severe psychotic symptoms. Individuals who reported psychotic symptoms at baseline were also more likely to develop schizophrenia if they used cannabis than were individuals who did not. The attributable risk of cannabis to psychosis was estimated at 13% for psychotic symptoms and 50% for cases with psychotic disorders that required psychiatric treatment.

Henquet *et al.* (2005) studied the relation between cannabis use and psychotic symptoms in individuals at risk for psychosis who first used cannabis during adolescence.

They tracked 2437 subjects (14–24 years) with and without risk for psychosis from the general population for 4 years and found a dose-dependent increased risk of psychosis in subjects exposed to cannabis (Henquet *et al.*, 2005). Interestingly, predisposition to psychosis was not found to be a predictor of future cannabis use at 4-year follow up. Adding to these studies, Stefanis *et. al.* (2004) reported that both positive and negative symptoms can be induced by cannabis consumption and are independent of each other. Finally, a number of cohort studies have reported a dose–response relationship in the increased risk of psychosis with cannabis exposure (Ferdinand *et al.*, 2005; Fergusson *et al.*, 2003; Henquet *et al.*, 2005; Stefanis *et al.*, 2004; van Os *et al.*, 2002; Weiser *et al.*, 2002).

Collectively, these epidemiological studies suggest that cannabis use may confer a nearly twofold higher risk for developing schizophrenia. This increased risk is comparable to the better known associations, such as the risk conferred by cigarette smoking in the development of lung cancer and the risk of heart disease from hypercholesterolemia.

Temporal relationship between cannabis use and the onset of schizophrenia: The onset of cannabis use may precede, follow, or co-occur with the onset of schizophrenia. However, schizophrenia begins insidiously, and evolves through several identifiable stages with the emergence of psychotic symptoms as the final step in the evolution of the disorder. As a result, while it maybe easy to pinpoint the emergence of positive psychotic symptoms in retrospective studies, pinpointing the onset of the less obvious prodromal symptoms is extremely challenging. Thus, while there is evidence suggesting a temporal association between cannabis use and the onset of positive psychotic symptoms, the temporal relationship between cannabis use and the less obvious symptoms has not been studied. Further, if as the neurodevelopmental hypothesis posits, that the pathophysiological processes underlying the illness precede the clinical manifestations by years or even decades and that these processes may even begin *in utero*, then, the argument about a temporal relationship is no longer relevant.

Nevertheless, there are a few studies that have systematically investigated the temporal order of cannabis use and the onset of schizophrenia. Allebeck and colleagues reported that in 69% of a schizophrenic patient sample from a Swedish case registry ($n = 112$), cannabis abuse preceded the onset of psychotic symptoms by at least 1 year (Allebeck *et al.*, 1993b). Further, in only 11% did the onset of psychotic symptoms precede the onset of cannabis abuse. Similarly, Linszen *et al.* (1994) found that cannabis abuse preceded the onset of psychotic symptoms by at least 1 year in 23 of 24 cannabis-abusing recent onset schizophrenic patients. Hambrecht and Hafner (1996, 2000) in their study of first-episode schizophrenic patients found that 14.2% of the sample had a lifetime history of drug abuse with cannabis being the most frequently abused drug (88%). Furthermore, drug abuse preceded the first sign of schizophrenia by more than a year but typically by more than 5 years in 27.5% of patients. In 37.9% of individuals, drug abuse followed the first sign of schizophrenia, and in 34.6% of individuals the first sign of schizophrenia and drug abuse started within the same month.

Effects of cannabis in individuals at high risk for developing schizophrenia: Another way to assess the risk of psychosis conferred by cannabis exposure is by studying the effects of cannabis in individuals at high risk for developing schizophrenia. In the Edinburgh High Risk study, individuals with a high genetic risk of schizophrenia, as evidenced by two or more affected relatives, cannabis use increased the risk for psychosis (Miller *et al.*, 2001). Furthermore, frequent cannabis use conferred a sixfold higher risk of schizophrenia in individuals with a family history of schizophrenia. In contrast, cannabis use or dependence in the previous year was not associated with a heightened risk of developing psychosis over the following 12-month period in a group of individuals at ultrahigh risk for developing schizophrenia (Phillips *et al.*, 2002). While the authors concluded that cannabis use did not appear to contribute to the onset of psychosis, they acknowledged

several limitations to the study design, including a low level of cannabis use in the sample and the lack of monitoring of cannabis use.

Cannabis is associated with an earlier onset of psychotic symptoms in schizophrenia: Some studies suggest that cannabis and other substance use is associated with an earlier age of and more abrupt onset of psychotic symptoms in schizophrenic patients (Addington and Addington, 1998; Allebeck *et al.*, 1993a; Andreasson *et al.*, 1987, 1989; Cleghorn *et al.*, 1991; Green *et al.*, 2004; Hambrecht and Hafner, 1996; Linszen *et al.*, 1994; McGuire *et al.*, 1994; Van Mastrigt *et al.*, 2004; Veen *et al.*, 2004).

Parallels in the association between amphetamines and psychosis and cannabis and psychosis: At this juncture, it would be illustrative to review an older but relevant story about the association between cannabis and psychosis. As early as 1938, Young and Scoville first reported an association between amphetamine use and psychosis. Nearly 20 years later, in a seminal report of 42 cases, Connell (1958) reported that high-dose amphetamine use by amphetamine addicts was associated with a florid psychosis. Despite some supporting data (dose–response, temporal association, and phenomenological similarities), there was considerable skepticism about the suggested association between amphetamines and psychosis as reviewed in (D'Souza *et al.*, 1999). First, it was not possible to determine whether amphetamine precipitated latent psychosis or *de novo* psychosis. Second, at the time "thought disorder," which was not a prominent feature described in early reports of amphetamine psychosis, was believed to be fundamental to and diagnostic of schizophrenia in American Psychiatry. Third, the prevailing diagnostic criteria of schizophrenia were poorly defined. Fourth, the role of sleep deprivation induced by amphetamines, in the development of amphetamine psychosis could not be excluded. Finally, it was unclear whether other drugs (sedative hypnotics, marijuana, and hallucinogens) taken along with amphetamines may have contributed to the development of psychosis in amphetamine abusers reviewed in (D'Souza *et al.*, 1999).

Prospective, controlled pharmacological studies with amphetamine provided critical support for the early DA hypothesis by addressing the limitations of naturalistic studies. In a series of studies, amphetamine loading was shown to induce psychosis in nonschizophrenic volunteers that spontaneously resolved (Angrist and Gershon, 1970; Angrist *et al.*, 1971; Bell, 1965, 1973; Griffith *et al.*, 1972). Drawing a parallel, pharmacological studies with cannabinoids address some of the limitations of naturalistic data similar to pharmacological studies with amphetamines. In particular, pharmacological studies offer the advantages of providing more accurate dose–response data, a sample carefully screened for preexisting illness, a more precise estimation of temporality and control of various confounds. While there are several reports of pharmacological studies with cannabinoids in humans, most of the studies were not specifically designed to study psychosis.

IV. Pharmacological Studies

In order to better interpret the pharmacological studies, it would be essential to understand some of the pharmacokinetic issues relevant to cannabis and Δ-9-THC. The pharmacokinetics and effects of Δ-9-THC vary as a function of route of administration. Herbal cannabis and cannabinoid compounds are typically consumed during recreational use by the inhaled or oral route. However, cannabinoids have also been administered for therapeutic or experimental purposes by the intravenous, rectal, sublingual, transdermal, topical (eye drops), and aerosolized route. Δ-9-THC administered by inhalation results in peak plasma concentrations within minutes, with psychotropic effects starting within seconds to a few minutes, reaching a peak after 15–30 min, and then tapering off within 2–3 h (Fig. 3). In attempting to quantify the dose of Δ-9-THC extracted from a typical cannabis joint several factors need to be considered including, but not limited to, the weight of a cannabis joint, the potency of Δ-9-THC in the herbal cannabis preparation, and the presence of other cannabinoids in herbal cannabis that might contribute to the effects of cannabis and/or alter the effects of Δ-9-THC (Karniol and Carlini, 1973; Karniol *et al.*, 1974, 1975; Turner *et al.*, 1980). Further, the amount of THC delivered is influenced by several factors, including the rate of inhalation, depth of puffs, duration of puffs, volume inhaled, extent of breath-holding after inhalation,

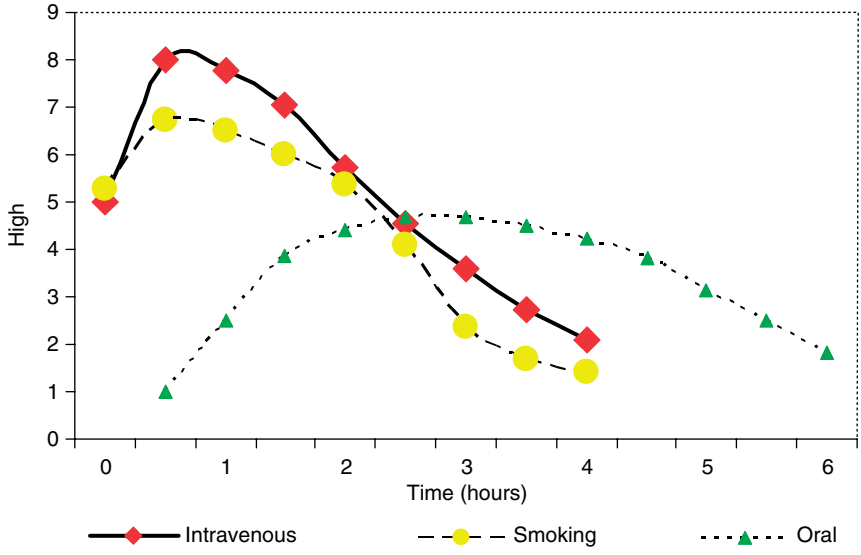

FIG. 3. Time course of subjective effects according to route of administration.

amount lost by smoke escaping into the air or respiratory dead space, vital capacity, length of cigarette smoked, adeptness of smoking, and subject's overall experience in titrating the dose. In fact, only 10–25% of the Δ-9-THC content of a cannabis joint enters the circulation when smoked (Adams and Martin, 1996). Thus, quantifying the typical dose of Δ-9-THC that a typical cannabis joint delivers is not without challenges. Intravenous dosing follows the pharmacokinetics of the inhaled route, though blood levels tend to be higher. Following oral ingestion, psychotropic effects set in with a delay of 30–90 min, reach their maximum after 2–3 h, and last for about 4–12 h (Hollister *et al.*, 1981; Ohlsson *et al.*, 1980, 1981). Nabilone is administered by oral route while levonantradol is administered by intramuscular route.

Psychosis associated with the consumption of medicinal cannabinoids: Cannabinoids including cannabis, natural and synthetic Δ-9-THC, nabilone, and levonantradol have been used in the treatment of chemotherapy-induced nausea, spasticity in multiple sclerosis, pain syndromes, glaucoma, stimulation of appetite, Tourette's syndrome, parkinsonism, dyskinesia, and traumatic brain injury. Adverse events causally linked to Marinol that occurred at >1% in the clinical trials included hallucinations, abnormal thinking, paranoid reaction, amnesia, and so on, all of which are symptoms of psychosis (Marinol Product monograph). Further, the incidence of "disturbing" psychiatric reactions increased with dose escalation. Similarly, studies with oral and intramuscular levonantradol have reported "loss of control," hallucinations, other perceptual alterations, thought disturbance, feelings of unreality, fear and paranoia, apprehension, difficulty concentrating, dissociation, depersonalization, dysphoria, anxiety, and panic (Citron *et al.*, 1985; Cronin *et al.*, 1981; Diasio *et al.*, 1981; Heim *et al.*, 1981, 1984; Jain *et al.*, 1981; Kenny and Wilkinson, 1982; Laszlo *et al.*, 1981; Sheidler *et al.*, 1984; Stuart-Harris *et al.*, 1983). Psychotropic adverse effects increased both with increasing dose and with repeated dosing (Citron *et al.*, 1985; Stambaugh *et al.*, 1984). Further, some subjects refused further testing because of the disturbing psychotropic effects. Nabilone (CESAMET^{TM}) was developed by Eli Lilly and marketed in Europe as an analgesic agent. A "toxic psychosis" has been reported as one of its side effects.

In a systematic review of randomized controlled trials comparing the antiemetic effects of cannabinoids with placebo or other antiemetics, 6% of patients receiving cannabinoids presented with hallucinations and 5% with "paranoia," while no patient treated with control drugs presented with such side effects (Tramer *et al.*, 2001). The same group conducted a systematic review of randomized controlled trials comparing the analgesic effects cannabinoids with placebo or other analgesics (codeine and benzopyranoperidine), but they did not specifically mention psychotic symptoms (Campbell *et al.*, 2001).

Experimental studies: There are a small number of pharmacological studies that were specifically designed to examine the behavioral and/or cognitive effects of

cannabinoids. As far back as the 1940s, pharmacological investigations were conducted under the direction of the "LaGuardia Committee on Marihuana" (Mayor's, 1944). With cannabis doses of about 30–50 mg (oral) and 8–30 mg (smoked), 12.5% of subjects experienced psychotic reactions. However, these subjects were prisoners and their mental status cannot be presumed to be healthy. Ames (1958) studied the effects of unassayed oral doses of cannabis extract (about 50–70 g Δ-9-THC) in 12 medical house staff who were presumably healthy. Subjects reported immediate recall deficits, thought fragmentation, dissociation between thoughts and action, disturbed temporal and spatial perception, visual illusions and hallucinations, derealization and depersonalization, mood alterations, and anxiety. Some subjects reported delusions of the presence of hidden recorders, fear of being hypnotized, fear of ECT, and fear of developing schizophrenia. One subject refused to answer questions for fear of being certified as insane. Isbell and colleagues (1967) studied the effects of varying doses of Δ-9-THC (120–480 μg/kg orally and 50–250 μg/kg smoked) in 40 former opiate addicts. At Δ-9-THC 120 μg/kg orally and 50 μg/kg smoking, in addition to recognizing the effects as being similar to marijuana, the subjects reported alterations in visual, auditory, and time perception. However, at Δ-9-THC doses of 300–480 μg/kg orally and 200–250 μg/kg by smoking there were marked auditory and visual distortions, depersonalization, derealization, and hallucinations. Of note, "occasional" individuals experienced psychosis even at low doses of Δ-9-THC. In a related study, Isbell and Jasinski (1969) compared the effects of Δ-9-THC (75–225 μg/kg, smoked) and LSD (0.5–1.5) in 10 "normal" controls. Both drugs produced perceptual distortions, mood changes, and at higher doses hallucinations. Of note, two subjects dropped out from the study after experiencing psychotic "reactions" from Δ-9-THC. However, Hollister showed that Δ-9-THC was not associated with as prominent psychotomimetic effects as LSD (reviewed in Hollister, 1986).

Melges *et al.* (1970) in a double-blind, placebo-controlled study with high and low dose Δ-9-THC reported that cannabis users were noted to have core symptoms of psychosis, including thought disorder and paranoia. The authors specifically described "tracking difficulties" that subjects reported, including racing thoughts, thought blocking, losing their train of thought, and so on. Jones and Stone (1970) did not observe robust psychotomimetic effects in studies of "normal" controls with Δ-9-THC (20 mg smoked or 40 mg orally). However, a few subjects reported ideas of reference and delusions that the researcher was using secret (unexplained) tests and hidden recording devices. At doses higher than 20 mg smoked or 40 mg orally, psychotomimetic effects including delusions, loosening of associations, and marked illusions began to emerge. In a ^{18}F-2-fluoro-2-deoxyglucose positron emission tomography (FDG-PET) study of intravenous Δ-9-THC (2 mg) on regional brain metabolism, two of eight healthy subjects who occasionally used cannabis, experienced paranoid-anxious reactions (Volkow *et al.*, 1991).

The pharmacological studies discussed thus far had several limitations, including the absence of placebo/control, lack of a double-blind, inclusion of psychiatrically ill individuals, and lack of standardized measures of psychosis. Recently, there have been a few laboratory studies examining the psychotogenic effects of cannabinoids that address some of these limitations.

Leweke *et al.* (1999b) reported the effects of synthetic Δ-9-THC (120 μg/kg) by oral route in 17 healthy individuals under controlled laboratory conditions. The study included subjects with past experience but no recent consumption of cannabinoids. The overall lifetime consumption of cannabinoids was limited to 10 times to exclude the long-term effects of cannabis use. Subjects with a history of recurrent abuse of illicit drugs other than cannabinoids or other psychiatric disorders were excluded. The primary outcome measure was binocular depth perception which has described as a model of illusionary perception. While the study was not placebo controlled, subjects were told that they might receive a placebo or active drug, but in fact they always received active drug. Subjective reactions ranged from mild euphoria to more pronounced reactions, including feelings of loss of self-control and body distortion suggestive of psychotic-like symptoms. One subject experienced a transient psychotic episode described as "a paranoid psychotic state with persecutory delusions, delusions of thought insertion, attentional irritability, fear, and—to some extent—verbal aggressive behavior." These symptoms resolved spontaneously within minutes to hours. Leweke *et al.* (2000) repeated the study with nabilone, a synthetic analogue of Δ-9-THC, and observed effects on binocular depth inversion similar that of Δ-9-THC (Leweke *et al.*, 2000).

D'Souza *et al.* (2004) characterized the behavioral and cognitive effects of Δ-9-THC in a double-blind, placebo-controlled study of healthy controls ($n = 22$). Only subjects with past cannabis experience but without lifetime cannabis abuse or dependence were included. Healthy subjects also underwent a Structured Clinical Interview for DSM-IV (healthy) and an unstructured psychiatric evaluation. Subjects were excluded if they had any significant psychiatric disorder and/or a family history of any DSM Axis I disorder. Subjects received in random order 5 or 2.5 mg of Δ-9-THC, or vehicle by intravenous route over 2 min. Positive and negative symptoms of psychosis were measured using the Positive and Negative Syndrome Scale (PANSS). Perceptual alterations that did not quite meet the threshold of psychosis were measured using the Clinician Administered Dissociative Symptoms Scale (CADSS). Cognitive symptoms were measured using tests of immediate recall (learning) and delayed recall, verbal fluency, working memory, and vigilance and distractibility. Δ-9-THC produced transient positive symptoms (Fig. 4), perceptual alterations, negative symptoms, euphoria, anxiety, and deficits in working memory and verbal recall, and the executive control of attention without altering general orientation.

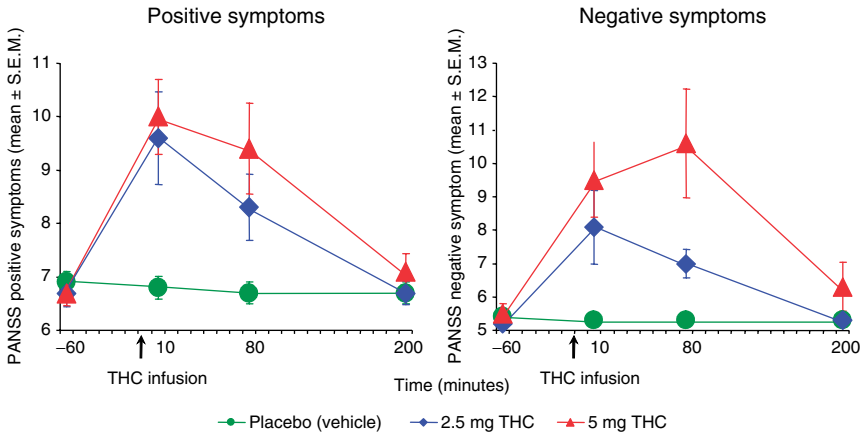

FIG. 4. Δ-9-THC induces positive and negative symptoms in healthy individuals; Positive and Negative Syndrome Scale.

Positive symptoms and perceptual alterations: The positive symptoms induced by Δ-9-THC included suspiciousness, paranoid and grandiose delusions, conceptual disorganization, and illusions. For example, healthy controls reported suspiciousness such as "I thought you all were *trying to trick me* by changing the rules of the tests to make me fail. I thought you were turning the clock back to confuse me," or "I thought that this was real …. I was convinced this wasn't an experiment," or "I thought you all were giving me THC thru the BP (blood pressure) machine and the sheets." Healthy controls also reported conceptual disorganization such as "I couldn't keep track of my thoughts … they'd suddenly disappear," or "It seemed as if all the questions were coming to me at once … everything was happening in staccato," or "my thoughts were fragmented… the past present and future all seemed to happening at once." Healthy subjects also reported unusual thoughts such as "I thought you could read my mind, that's why I didn't answer …. I felt as if my mind was nude," or "I felt I could see into the future …. I thought I was God." These effects reported by carefully screened healthy subjects appear to be remarkably similar to the kinds of psychotic symptoms reported by patients with schizophrenia.

An identical study was conducted in parallel in medicated schizophrenic patients. Δ-9-THC *transiently* exacerbated a range of positive and negative symptoms, perceptual alterations, cognitive deficits, and medication side effects associated with schizophrenia without producing any obvious "beneficial" effects. The increases in psychosis were brief, modest, and occurred even though subjects were clinically stable, medication-responsive, and were receiving therapeutic

doses of antipsychotics. The positive symptoms induced in these patients were similar to their typical symptoms. Using a threshold score of clinically significant positive symptoms (PANSS positive symptom subscale score $\geq$3 points) defined *a priori*, schizophrenia patients appeared to be more sensitive to the Δ-9-THC effects. Eighty percent of the schizophrenia group but only 35% of controls had a suprathreshold response to 2.5 mg Δ-9-THC and 75% of schizophrenic patients but only 50% of controls had a suprathreshold response to 5 mg Δ-9-THC (Fig. 5). Similarly, (Lindeman and Malamud 1934) administered unassayed doses of hashish to a group of schizophrenic patients, "neurotics," and normals. Normal individuals developed paranoid delusions, impulsivity, and marked perceptual changes and schizophrenic patients experienced an exacerbation of symptoms (Lindemann and Malamud, 1934).

Perceptual alterations: Δ-9-THC also produced depersonalization, derealization, distorted sensory perceptions, altered body perception, feelings of unreality, and extreme slowing of time in both healthy individuals and patients with schizophrenia (Fig. 6). Subjects were reported as being "spaced out," looking "separated or detached," and as if they said or did "something bizarre," or if they needed redirection.

Negative symptoms: Δ-9-THC produced negative symptoms in healthy individuals which included blunted affect, reduced rapport, lack of spontaneity,

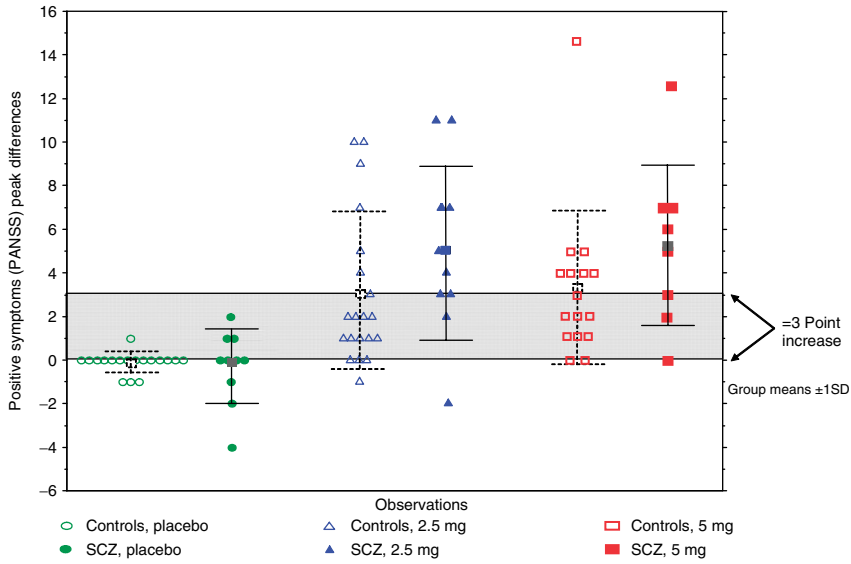

FIG. 5. Enhanced sensitivity to the psychotomimetic effects of d-9-THC in schizophrenia.

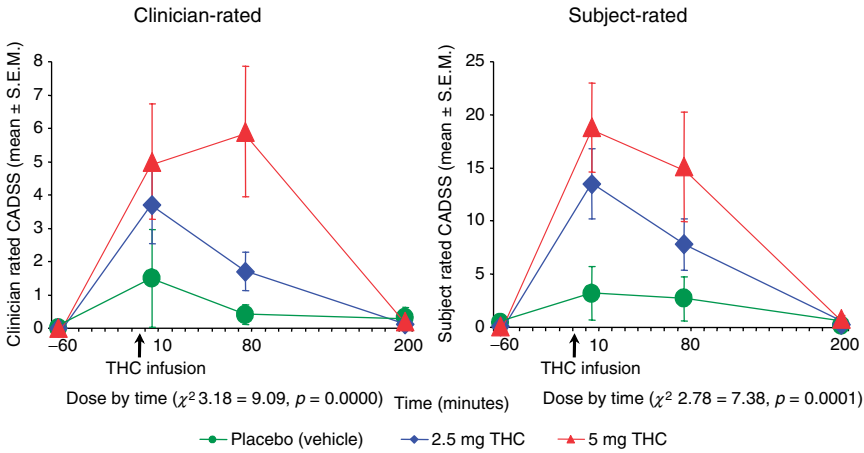

FIG. 6. Δ-9-THC induces transient perceptual alterations in healthy individuals; Clinician Administered Dissociative Symptoms Scale.

psychomotor retardation, and emotional withdrawal (Fig. 4). How much sedation and/or being inwardly preoccupied contributed to the increased ratings of negative symptoms was unclear. These schizophrenia-like negative symptoms may have been confounded by the known cataleptic and sedating effects of Δ-9-THC. Besides, acute pharmacological studies may have limitations in their capacity to "model" negative symptoms. Finally, Δ-9-THC transiently increased negative symptoms in schizophrenic patients.

Of note, a persistent "amotivational syndrome" has been described in chronic heavy cannabis users by some (Halikas *et al.*, 1982; Hall and Solowij, 1998; Kolansky and Moore, 1971b; Millman and Sbriglio, 1986; Tennant and Groesbeck, 1972b) but not others (Carter *et al.*, 1980; Hollister, 1988; Rubin and Comitas, 1975). This so-called "amotivational syndrome" is characterized by apathy, amotivation, social withdrawal, narrowing of interests, lethargy, impaired memory, impaired concentration, disturbed judgment, and impaired occupational achievement. The syndrome has a striking phenomenological similarity with the negative dimension of psychosis and appears to be dose related. However, other drug use, poverty, low socio-economic status, or preexisting psychiatric disorders existing data may confound the interpretation of the existing literature.

Cognitive deficits: The most consistent acute cognitive effects of cannabinoids in humans include deficits in learning, short-term memory, working memory, and attention (Hart *et al.*, 2001; Heishman *et al.*, 1990; Hooker and Jones, 1987; Leweke *et al.*, 1998; Marks and MacAvoy, 1989; Miller *et al.*, 1977). These are also the cognitive deficits observed in schizophrenia (Heinrichs and Zakzanis, 1998). Of note, the most robust effects of cannabinoids are on verbal memory

reviewed in Ranganathan and D'Souza (2006), the latter is also the most robust cognitive deficit observed in schizophrenia (Heinrichs and Zakzanis, 1998).

In healthy subjects (hatched lines), Δ-9-THC significantly impaired immediate free recall in a dose-dependent manner across all three trials of immediate recall (Fig. 7). Δ-9-THC also impaired delayed (+30 min) free recall and delayed cued recall in a significant, dose-dependent manner. The effect on delayed recognition recall trended toward significance. Finally, Δ-9-THC increased the number of false positives and intrusions with a trend toward significance. Relative to controls, schizophrenia patients were specifically more vulnerable to the dose-related learning impairments produced by Δ-9-THC (D'Souza *et al.*, 2005). Under the influence of 5 mg Δ-9-THC schizophrenia patients (solid lines) showed no learning whatsoever (Fig. 7). Δ-9-THC also increased the number of intrusions and false positives generated during recall. Further, 5 mg Δ-9-THC reduced learning and recall in healthy controls to the level of schizophrenia patients on the placebo condition.

While the acute transient effects of cannabinoids on memory are quite clear, whether cannabinoids produce impairments in memory that persist beyond the period of intoxication, remains inconclusive. Heavy and prolonged cannabis exposure may be associated with deficits in memory, sustained attention, and executive functioning (Bolla *et al.*, 2002; Pope and Yurgelun-Todd, 1996; Pope *et al.*, 1995; Solowij, 1995; Solowij *et al.*, 1995, 2002). Eleven of 22 (50%) studies

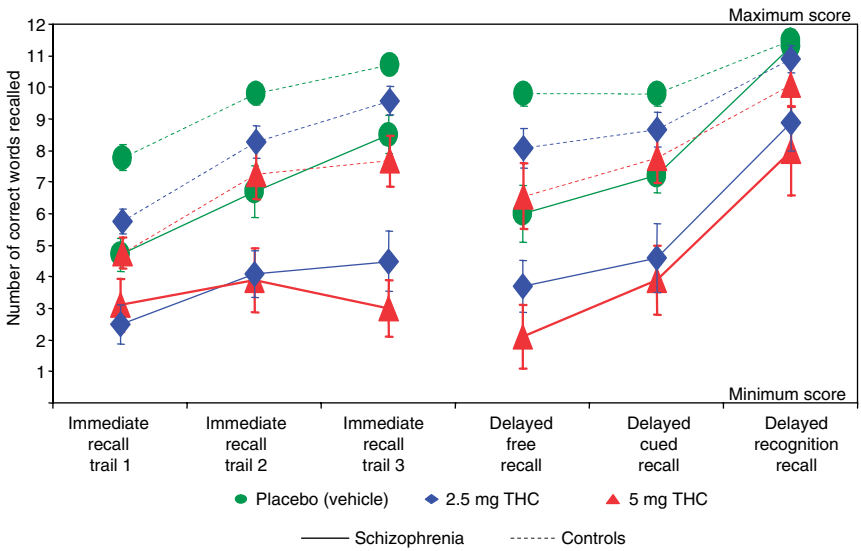

FIG. 7. Δ-9-THC induces memory impairments; Hopkins Verbal Learning Test.

involving clinical samples (cannabis abusers) and 5 of 7 (75%) population-based samples support an association between cannabis use (excluding intoxication) and cognitive deficits. With a few exceptions, the literature has limitations including (1) the lack of any measures of cognitive functioning prior to onset of cannabis use, (2) small samples (range 9–63; median = 26), (3) selection bias, (4) confound of other drug and/or alcohol use, and (5) sensitivity of the cognitive measures, and so on.

In a meta-analysis of 15 studies, Gonzalez *et al.* (2002) concluded that a majority of studies found evidence for persistent but subtle cognitive deficits associated with nonacute (remote) cannabis use. However, whether these cognitive deficits are reversible and persist despite cessation of cannabis use remains an open question. In the first published report examining whether the residual cognitive effects of cannabis persist beyond a period of abstinence longer than 12–72 h, Pope *et al.* (2001) found that deficits in cognitive test performance in cannabis abusers that were present at 7 days normalized by day 28 (Pope *et al.*, 2001). In contrast, Bolla *et al.* (2002) found that cognitive test performance in cannabis abusers with a history of very heavy use of cannabis showed persistent decrements in learning and recall, executive function, psychomotor speed, complex reaction time, and manual dexterity even after 28 days of abstinence. The magnitude of the difference in mean performance between the heavy and light users was between 1.0 and 3.3 S.D. units (Bolla *et al.*, 2002). Similarly, the magnitude of the association between cannabis use and decreasing Wisconsin Card Test Performance (executive functioning) was large (4.1–4.2 S.D. units).

The question of enduring deficits associated with cannabis abuse are only beginning to be investigated with more sensitive measures such as brain imaging and electrophysiology. Cannabis abusers have altered regional cerebral blood flow (rCBF) at rest in several brain regions even after 26 h of monitored abstinence (Block *et al.*, 2000). Similarly, Block *et al.*, have shown decreases in memory-related blood flow in prefrontal cortex in frequent cannabis users in the unintoxicated state relative to nonusers, increases in memory-relevant regions of cerebellum, and altered lateralization in hippocampus. The speed of information processing, measured by the latency of parietal P300, was delayed significantly with increasing frequency of use (Solowij *et al.*, 1995). In summary, there is some overlap between the cognitive effects of cannabinoids and the cognitive deficits observed in schizophrenia.

Δ-9-THC produces a plethora of effects including euphoria, a calm and relaxed feeling state, psychotomimetic symptoms, tachycardia, etc. in the absence of any change in orientation. While some but not other studies reviewed suggest that cannabis can induce a broad range of transient effects in healthy individuals that share some similarities with some, though not all, of the symptoms of schizophrenia, the data from these acute studies do not, however, address the

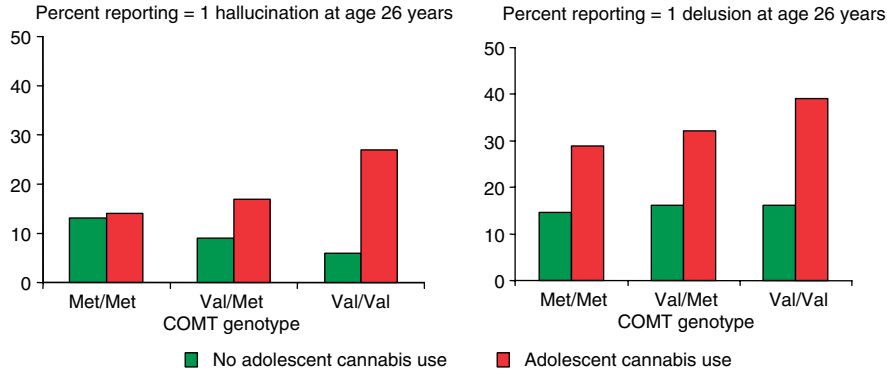

FIG. 8. COMT gene polymorphism moderates influence of adolescent cannabis use on developing psychosis in adulthood.

question of whether cannabis can cause a chronic psychotic disorder such as schizophrenia, that persists through life.

What puts some individuals at higher risk for experiencing psychotic symptoms following exposure to cannabinoids? Individuals with a vulnerability to psychosis as estimated by a psychosis proneness scale were more likely to report psychotic symptoms with cannabis use (Verdoux *et al.*, 2003b). In a preliminary report, no structural mutations in CB1R were found in individuals who developed acute psychotic symptoms after cannabis intake (Hoehe *et al.*, 2000). Caspi *et al.* (2005) reported that a polymorphism of the catechol-*O*-methyl transferase (COMT) gene modulates the risk of schizophrenia conferred by cannabis (Fig. 8). After adolescent exposure to cannabis, individuals homozygous for the COMT valine158 allele were most likely to exhibit psychotic symptoms and to develop schizophreniform disorder later. The effects of cannabinoids on dopamine function may be involved in this gene by cannabis interaction.

V. Cannabis and Psychosis: Causality

Some of the criteria that have been used to establish disease causality include temporality, strength of the association, direction, dose–response or biological gradient, consistency, specificity, coherence, strength of the relationship, experimental evidence, and biologic plausibility (Aiello and Larson, 2002; Hill, 1965).

Most studies provide evidence of direction by showing that the association between cannabis use and psychosis persists even after controlling for many potential confounding variables such as gender, age, ethnicity, low IQ, level of

education, urbanicity, disturbed behavior, cigarette smoking, poor social integration, unemployment, single marital status, and previous psychotic symptoms. While there is a strong association between cigarette smoking and schizophrenia, there is little evidence to support the notion that cigarette smoking "causes" schizophrenia. In contrast, the high rates of cigarette smoking in schizophrenia may reflect an attempt by schizophrenia patients to self-medicate deficits in information processing. Several studies reviewed here provide evidence of a dose–response relationship between cannabis exposure and the risk of psychosis. With regard to temporality, some, though not all, studies suggest that cannabis use precedes or coincides with the onset of psychosis in about two-third of patients with schizophrenia who use cannabis. Related to this, cannabis use may be associated with an earlier age of onset of schizophrenia. Further, there is also evidence that the earlier the onset of cannabis use, the stronger the effect on schizophrenia outcomes. There is also some evidence for both the relative specificity of exposure (i.e., cannabis) and specificity of the outcome (i.e., psychosis). Experimental evidence from laboratory studies suggests that cannabinoids can induce a wide range of transient schizophrenia-like symptoms in healthy individuals and relative to controls, schizophrenia patients are more vulnerable to the psychotomimetic effects of Δ-9-THC. Whether exposure to cannabis can result in a chronic psychotic state that persists beyond the period of intoxication is unclear.

However, not all patients with psychosis have been exposed to cannabis and not all cannabis users develop psychosis. Furthermore, there is a disparity in the incidence and prevalence of cannabis abuse (7–12%) and that of schizophrenia (1–2%), and despite different rates of cannabis consumption across the globe, there is relative uniformity in the incidence of schizophrenia. Further, the increase in cannabis use and the use of more potent forms of cannabis in certain geographical areas has not been accompanied or followed by a commensurate increase in the rates of schizophrenia (Degenhardt *et al.*, 2003). Similarly, if cannabis use is associated with an earlier age of onset, then the increase rates of cannabis use should result in a trend toward a lower age of onset of schizophrenia. This does not seem to be the case.

Taken collectively, it appears that cannabis is neither a necessary nor a sufficient cause of schizophrenia. Similarly, cigarette smoking is neither a necessary nor a sufficient cause of lung cancer. More likely, cannabis exposure is a component or contributing cause which interacts with other known, for example, genetic and heretofore unknown factors, leading to schizophrenia. In terms of strength, cannabis confers about a two- to threefold increase in the relative risk for schizophrenia. Arsenault *et al.* (2004) have suggested that the number of cases of schizophrenia in a population that could be eliminated by removal of cannabis use, the population attributable fraction (PAF), is about 8% (Arsenault *et al.*, 2004). In the absence of known causes of schizophrenia, the role of component causes such as cannabinoid exposure remains important and warrants further study.

As reviewed by Piomelli, advances in the understanding of brain cannabinoid receptor function now offer several biologically plausible mechanisms by which cannabis exposure might induce psychosis. While it is out of the scope of this chapter, the interactions between cannabinoid receptor function and dopamine, glutamate and GABA receptor function provide potential mechanisms by which cannabis contribute to the pathophysiology of psychosis reviewed in D'Souza *et al.* (2004, 2005).

VI. Cannabinoid Receptor Dysfunction and Psychotic Disorders

Emerging findings from postmortem (Dean *et al.*, 2001; Zavitsanou *et al.*, 2004), neurochemical (Giuffrida *et al.*, 2004; Leweke *et al.*, 1999a), and genetic (Ujike *et al.*, 2002) studies suggest that cannabinoid receptor system dysfunction that may contribute to the pathophysiology of schizophrenia.

Leweke *et al.* (1999a) and colleagues were the first to suggest altered cannabinoid receptor function in schizophrenia. Levels of anandamide, 2-AG, palmitoylethanolamide (PEA), and a noncannabinoid acylethanolamide, oleylethanolamide (OEA) as a positive control, were measured in cerebrospinal fluid (CSF) sampled from 10 schizophrenia patients and 11 healthy controls (Fig. 9). Mean anandamide and PEA levels were approximately twofold higher in the schizophrenia patients. These differences could not be attributable to drug use, and neither age, gender nor medication correlated with CSF endocannabinoid levels. However, since some subjects were neuroleptic naïve and others were not, it was not possible to fully exclude an effect of antipsychotic drugs.

These data were replicated in a larger sample and the confound of medication status was also addressed (Giuffrida *et al.*, 2004). CSF anandamide levels were eightfold higher in antipsychotic-naive first-episode paranoid schizophrenics than in healthy and psychiatric controls (Fig. 10). The elevations in CSF anandamide seen in antipsychotic naive schizophrenic patients were absent in schizophrenics treated with "typical" antipsychotics but not in those treated with "atypical" antipsychotics ($n = 34$). Finally, CSF anandamide levels negatively correlated with psychotic symptoms in unmedicated patients. Since anandamide release may serve as an inhibitory feedback signal countering dopamine activation, the increase in CSF anandamide levels in unmedicated acutely psychotic patients was interpreted as a compensatory increase in endocannabinoids secondary to psychosis-related hyperdopaminergia. While CSF studies of endocannabinoids have served to draw attention to possible endocannabinoid dysfunction in schizophrenia, they are not without limitations. Endocannabinoids are very challenging to assay. Anandamide has a very short half-life and therefore differences in collection and processing of samples might explain the group differences. Finally, CSF

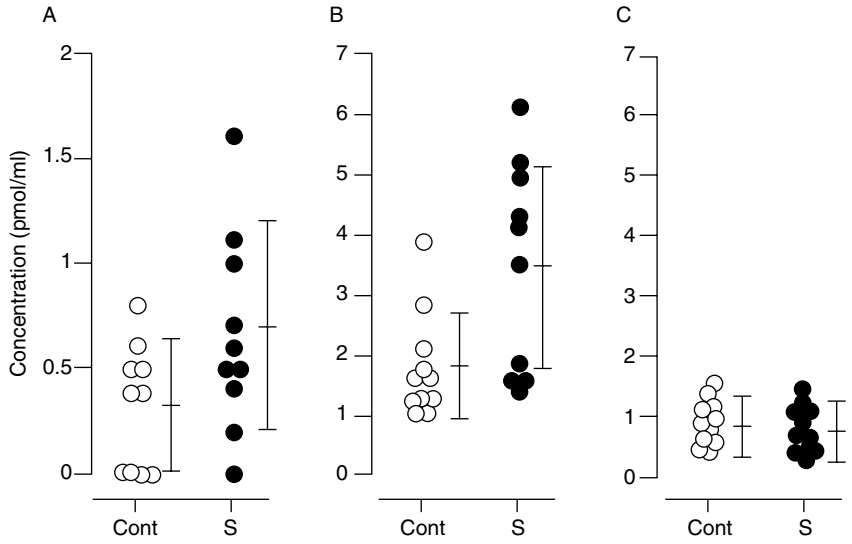

FIG. 9. Cerebrospinal fluid endocannabinoid levels in schizophrenia. Concentrations of anadamide (A), palmitylethanolamide (B), and OEA oleylethanolamide (C) in CSF of schizophrenic (S) and nonschizophrenic (Cont) subjects. Bars indicate mean ± S.D. for each group [(A) and (B), $p < 0.05$; Student's *t*-test]; Leweke *et al.* (1999).

endocannabinoid levels may reflect global rather than regional changes. Studies directly examining CB1 receptor may address some of these limitations.

There are two postmortem studies of CB1 receptor changes in schizophrenia in the literature. Compared with control subjects, schizophrenia patients showed a 19% increase ($p < 0.05$) in CB1 receptor density only in the dorsolateral prefrontal cortex (DLPFC) (Dean *et al.*, 2001). These differences could not be attributable to postmortem interval, brain pH, age, or gender. Further, there were no significant correlations between CB-1R binding and duration of illness or antipsychotic drug dose in those with schizophrenia. Of note, chronic antipsychotic drug treatment study in rats does not result in changes in CB1-receptor binding in the cerebral cortex, caudate-putamen (CP), or hippocampus (Sundram *et al.*, 2004). There were no significant differences between the groups in the CP or hippocampal formation. In subjects who had recently consumed cannabis, there was a significant ($p < 0.05$) 23% increase in CB1 receptor density in the CP compared to nonusers independent of schizophrenia. Zavitsanou *et al.* (2004) compared CB1 receptor in the anterior cingulated cortex (ACC) taken postmortem from patients with schizophrenia ($n = 10$) and matched control subjects ($n = 9$) using [^{3}H]SR141716A. Compared to the control group schizophrenia patients had a significant 64% increase in CB1 receptor density in the ACC. The effects of

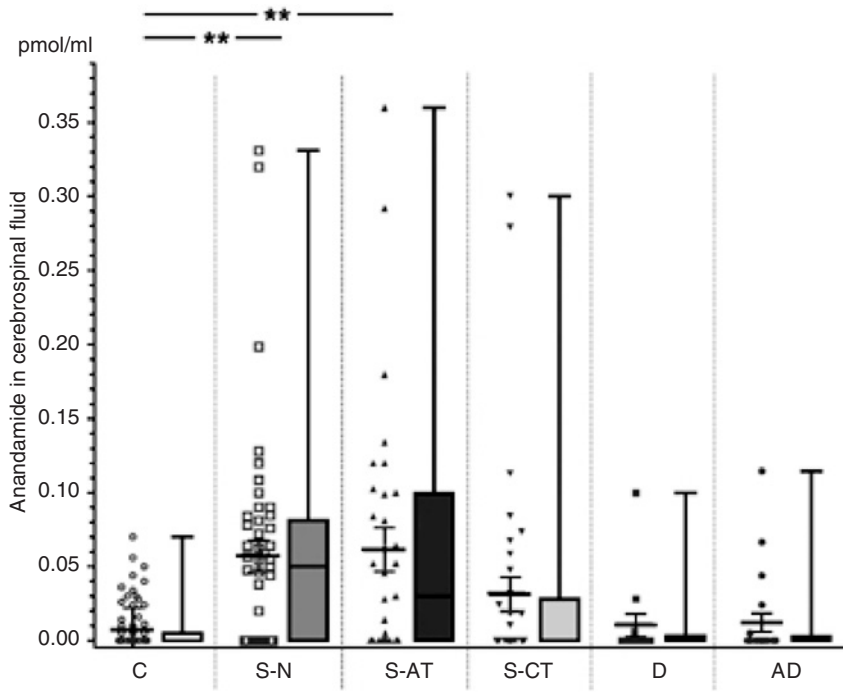

FIG. 10. Elevated anandamide in the CSF of antipsychotic-free first-episode schizophrenic patients. CSF anandamide levels in healthy volunteers (C); antipsychotic-free schizophrenics with paranoid schizophrenia (S-N); acute schizophrenics (paranoid type) on "atypical" (S-AT) or "typical" (S-CT) antipsychotic drugs; and patients with dementia (D) or affective disorders (AD) without psychotic symptoms. Single values with mean ± SEM as well as corresponding ox-plots illustrating median, range, and quartiles for each group. Statistically significant differences between groups are shown (*$p < 0.01$; **$p < 0.001$; Kruskal-Wallis test followed by Mann–Whitney test; a Bonferroni correction (5 tests) of the α-level was applied: $\alpha = 0.05/5 = 0.01$).

antipsychotic treatment or premorbid cannabis use explaining the group differences could not be ruled out. Together the postmortem studies provide some preliminary evidence suggestive of endocannabinoids dysfunction in schizophrenia. The development of good radioligands that permit *in vivo* imaging of the CB1 receptor in schizophrenia patients would help confirm the limited postmortem findings.

Finally, several groups have looked for associations between schizophrenia and genes relevant to cannabinoid receptor function. The CB1 receptor gene is mapped to chromosome 6q14–15, and linkage studies have produced evidence for a schizophrenia-susceptibility locus in this region. Two polymorphisms for the

CB1 receptor gene have been identified, a triplet repeat (AAT)n in the 3′-flanking region (Dawson *et al.*, 1995) and a biallelic silent mutation of 1359 G-to-A at the 453 codon in the coding exon (Gadzicki *et al.*, 1999). Dawson failed to show a significant association between the (AAT)n triplet repeat polymorphism of the CB1 receptor gene and schizophrenia in 135 schizophrenic subjects compared to 101 control subjects (Dawson, 1995). Similarly, Tsai replicated these findings in a study comparing 127 subjects with schizophrenia and 146 control subjects in a Han Chinese population (Tsai *et al.*, 2000). They concluded the (AAT)n triplet repeat in the promoter region of the CB1 receptor gene is not directly involved in the pathogenesis of schizophrenia in Chinese populations. In contrast, Ujike *et al.* (2002) reported that the (AAT)n triplet repeat polymorphism of the CB1 receptor gene was significantly associated with patients with schizophrenia, especially the hebephrenic subtype. The functional effect of this triplet repeat on the CB1 receptor gene transcription rate remains unclear. Leroy studied the 1359 A/G polymorphism in a French Caucasian sample of 102 subjects with schizophrenia and 63 healthy controls (Leroy *et al.*, 2001). Overall there were no significant differences between the two groups either in allele frequency or genotype distribution. However, when the patient group was divided into a substance using ($n = 42$) and nonusing, they found a significant decrease in homozygosity for the G allele in nonusers compared to users ($p < 0.04$). Fatty acid amide hydrolase (FAAH) is the primary catabolic enzyme of endocannabinoids. Morita *et al.* (2005) found no significant association of a nonsynonymous polymorphism (Pro129Thr) of the FAAH gene with schizophrenia.

VII. Summary and Conclusions

Cannabinoids can induce acute transient psychotic symptoms or an acute psychosis in some individuals. What makes some individuals vulnerable to cannabinoid-related psychosis is unclear. Cannabinoids can also exacerbate psychosis in individuals with an established psychotic disorder, and these exacerbations may last beyond the period of intoxication. Less clear is whether cannabis *causes* a persistent *de novo* psychosis. The available evidence meets many but not all the criteria for causality, including dose–response, temporality, direction, specificity, and biological plausibility. On the other hand, the large majority of individuals exposed to cannabinoids do not experience psychosis or develop schizophrenia. It is more likely that cannabis exposure is a component cause that interacts with other factors, for example, genetic risk, to "cause" schizophrenia. Nevertheless, in the absence of known causes of schizophrenia, the role of component causes such as cannabis exposure (exogenous hypothesis), is important and warrants further study. There is also tantalizing evidence from postmortem,

neurochemical, and genetic studies, suggesting CB1 receptor dysfunction (endogenous hypothesis) in schizophrenia that warrants further investigation. Further work is necessary to identify those factors that place individuals at higher risk cannabinoid-related psychosis, to identify the biological mechanisms underlying the risks and to further study whether CB1 receptor dysfunction contributes to the pathophysiology of psychotic disorders. Finally, from a treatment perspective, given the negative impact of cannabis use on the course and expression of schizophrenia, and the potential for precipitating psychosis in individuals at risk for schizophrenia, efforts need to be directed toward developing effective treatments for cannabis use disorders.

Acknowledgments

The author wishes to acknowledge support from the (1) Department of Veterans Affairs (Schizophrenia Biological Research Center), (2) National Institute of Mental Health (R01MH61019–02 to DCD), (3) National Institute of Drug Abuse (RO1DA12382–01), (4) Stanley Medical Research Institute, and (5) Donaghue Foundation.

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INVOLVEMENT OF NEUROPEPTIDE SYSTEMS IN SCHIZOPHRENIA: HUMAN STUDIES

Ricardo Cáceda, Becky Kinkead, and Charles B. Nemeroff

Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine
Atlanta, Georgia 30322, USA

Neuropeptides are heterogeneously distributed throughout the digestive, circulatory, and nervous systems and serve as neurotransmitters, neuromodulators, and hormones. Neuropeptides are phylogenetically conserved and have been demonstrated to regulate numerous behaviors. They have been hypothesized to be pathologically involved in several psychiatric disorders, including schizophrenia. On the basis of preclinical data, numerous studies have sought to examine the role of neuropeptide systems in schizophrenia. This chapter reviews the clinical data, linking alterations in neuropeptide systems to the etiology, pathophysiology, and treatment of schizophrenia. Data for the following neuropeptide systems are included: arginine-vasopressin, cholecystokinin (CCK), corticotropin-releasing factor (CRF), interleukins, neuregulin 1 (NRG1), neurotensin (NT), neuropeptide Y (NPY), opioids, secretin, somatostatin, tachykinins, thyrotropin-releasing hormone (TRH), and vasoactive intestinal peptide (VIP). Data from cerebrospinal fluid (CSF), postmortem and genetic studies, as well

INTERNATIONAL REVIEW OF NEUROBIOLOGY, VOL. 78
DOI: 10.1016/S0074-7742(06)78011-4

0074-7742/07 $35.00

as clinical trials are described. Despite the inherent difficulties associated with human studies (including small sample size, variable duration of illness, medication status, the presence of comorbid psychiatric disorders, and diagnostic heterogeneity), several findings are noteworthy. Postmortem studies support disease-related alterations in several neuropeptide systems in the frontal and temporal cortices. The strongest genetic evidence supporting a role for neuropeptides in schizophrenia are those studies linking polymorphisms in NRG1 and the CCK_A receptor with schizophrenia. Finally, the only compounds that act directly on neuropeptide systems that have demonstrated therapeutic efficacy in schizophrenia are neurokinin receptor antagonists. Clearly, additional investigation into the role of neuropeptide systems in the etiology, pathophysiology, and treatment of schizophrenia is warranted.

I. Introduction

Elucidation of the etiology and pathophysiology of schizophrenia with the goal of developing novel prevention and treatment approaches has included examination of the role of neuropeptide systems. Neuropeptides, often referred to as gut–brain peptides because of their high concentrations in both tissues, regulate numerous behaviors (Bennett *et al.*, 1997; Ramirez *et al.*, 2004) and have been implicated in the pathophysiology of several major psychiatric diseases (Holsboer, 2003; Inui, 2003; Kinkead and Nemeroff, 2004; Nemeroff and Vale, 2005). Although neuropeptides clearly function as neurotransmitters (directly modifying the electrical state of neurons), they also function as neuromodulators (modifying the effects of other, primary neurotransmitters such as dopamine, glutamate, serotonin, and GABA) and as neurohormones. Elimination of a neuropeptide or neuropeptide receptor has a wide impact on normal animal behavior, ranging from no effect in the absence of an additional pharmacological or environmental challenge (Coste *et al.*, 2000; Ragnauth *et al.*, 2005; Sharpe *et al.*, 2005; Weninger *et al.*, 1999a,b) to a frankly abnormal phenotype (Bielsky *et al.*, 2005; Colwell *et al.*, 2003; Dauge *et al.*, 2001; Nishimori *et al.*, 1996; Yamada *et al.*, 2001). Despite the relatively subtle behavioral effects of neuropeptides, considerable preclinical data have implicated neuropeptide systems in schizophrenia, not the least of which is their widespread CNS distribution in brain regions implicated in this disorder and the well-documented effects of antipsychotic drugs on these circuits.

Overall, the clinical data implicating a preeminent role for one or another neuropeptide in schizophrenia is not strong, although the overall database is far smaller than the multitude of studies on dopamine and other monoamines. Factors contributing to this include the heterogeneity of schizophrenic patients, including sex, age, substance abuse, comorbid disorders, and chronic exposure to

antipsychotic drugs. However, several clinical studies have consistently associated dysregulation of neuropeptide systems with specific patient subgroups characterized by common symptomatology or treatment response (Breslin *et al.*, 1994; Garver *et al.*, 1991; Tachikawa *et al.*, 2000; Zhang *et al.*, 2000).

Moreover, inadequate methods for antemortem measurement of indices of the activity of neuropeptidergic systems in the human brain in patients contribute to our paucity of information on neuropeptides in schizophrenia. For example, the virtual absence of ligands to measure peptide receptor density with positron emission tomography (PET) or single photon emission computed tomography (SPECT) has limited antemortem studies to measurements of neuropeptides in the cerebrospinal fluid (CSF). However, although neuropeptides measured in the CSF are believed to originate largely but not completely from the CNS, the specific brain regions of origin and their relative contribution remain obscure. Although postmortem studies allow for a high degree of neuroanatomical resolution, results are subject to a number of potential confounds, including postmortem delay interval and chronic antipsychotic (and other) drug exposure, as well as effects of agonal state. An additional consideration in CSF and postmortem studies is the difficulty of obtaining samples from antipsychotic drug-free subjects.

In this chapter, the results of clinical studies examining the role of neuropeptide systems in schizophrenia are reviewed. The peptides discussed include arginine-vasopressin (AVP), cholecystokinin (CCK), corticotropin-releasing factor (CRF), interleukins, neurotensin (NT), neuropeptide Y (NPY), opioids, secretin, somatostatin, tachykinins, thyrotropin-releasing hormone (TRH) and vasoactive intestinal peptide (VIP). Table I summarizes the neuropeptides discussed in this chapter, identified receptor subtypes, and the available receptor agonists and antagonists. Data from CSF, postmortem and genetic studies, as well as clinical trials will be described.

II. Cholecystokinin

CCK was first isolated from the gastrointestinal tract in 1928 (Ivy and Oldberg, 1928) and was shown to stimulate pancreatic secretion and gall bladder contraction. CCK is heterogeneously distributed in the gastrointestinal, central, and peripheral nervous systems (for review see Miyasaka and Funakoshi, 2003). Several biologically active forms of CCK have been identified and share a common N-terminal sequence (CCK-4, CCK-8, CCK-22, CCK-33, CCK-39, and CCK-58), with CCK-8 and CCK-58 being the most abundant forms in the brain. The peptide gastrin (encoded on human chromosome 17) and CCK (encoded on human chromosome 3) have identical 6 N-terminal amino acids but exhibit different sulphation sites (Lund *et al.*, 1986). In the CNS, CCK is

TABLE I
General Properties of Neuropeptides Implicated in Schizophrenia

Peptide	Related peptides (number of aa)	Identified receptors	Agonists	Antagonists
CCK	Prepro-CCK	CCK_A	Cerulein (CCK_A,CCK_B)	Proglumide (CCK_A)
	(4, 8, 22, 33, 39, 58)	CCK_B	Gastrin (CCK_B)	Dexloxiglumide (CCK_A)
				Devazepide (CCK_A)
				L365,260 (CCK_B)
CRF	CRF (41)	CRF_1	Urocortins (CRF_2)	GSK 876008 (CRF_1)
	Urocortin I, II, III (40, 38, 38)	CRF_2,($sCRF_{2\alpha}$,		R121919 (CRF_1)
	Urotensin I, II, III (41, 11, 29)	$sCRF_{2\beta}$, $sCRF_{2\gamma}$)		
	Sauvagine (40)			
Dynorphins	Prodynorphin	κ Receptor	Ethylketocyclazocine (κ)	Norbinaltorphimine (κ)
	Dynorphin A (8, 17)		U50,488H (κ)	
	Dynorphin B (13)		U69,593 (κ)	
Endorphins	POMC	μ Receptor	Morphine (μ)	Naloxone (μ, κ, δ)
	-β-endorphin (30)		Fentanyl (μ)	Naltrexone (μ, κ, δ)
	-ACTH (39)		Levorphanol (μ)	Cyprodime (μ)
	-α-MSH (13)		Dermorphin (μ)	
	-α, -β, -γ endorphins			
Enkephalins	Proenkephalin	δ Receptor	Deltorphin (δ)	Naloxone (μ, δ, κ)
	-Leu-enkephalin (5)			Naltrexone (μ, δ, κ)
	-Met-enkephalin (5)			ICI 174864 (δ)

Gastrin	Gastrin (6)	CCK_B	JB93242 (CCK_B)	JB93182 (CCK_B)
NT	Prepro-NT/Neuromedin N	NT_1–NT_4	NT69L (NT_1)	SR 48692 (NT_1)
	-Neurotensin (13)		Eisai compound (NT_1)	SR 142948A (NT_1, NT_2)
	-Neuromedin N (6)		JMV 449 (NT_1)	
			PD149163 (NT_1)	
NPY	Pro-NPY	NPY_1–NPY_5	Pancreatic polypeptide	H-409/22 (NPY_1)
	-NPY(36)		Peptide YY	1229U91 (NPY_1)
Somatostatin	Prosomatostatin	SST_1–SST_5	Octeotride (SST_2)	CYN 154806 (SST_2)
	-Somatostatin (14, 28)		Seglitide (SST_2)	PRL-2903 (SST_2)
			Lanreotide (SST_2)	BIM-23056 (SST_5)
			Cortistatin (SST_1-SST_5)	
Tachykinins	Preprotachykinin I		GR73632 (NK_1)	Vestipitant (NK_1)
	-Substance P (11)	NK_1		Saredutant (NK_2)
	-Neurokinin A (10)	NK_2		Osanetant (NK_3)
	-Neurokinin B (10)	NK_3		Talnetant (NK_3)
	Preprotachykinin II			SSR 146977 (NK_3)
	-Neurokinin B (10)			
TRH	Pro-TRH	THR-R1	TA-0910	–
	-TRH (3)	THR-R2	DN-1417	
VIP	Prepro-VIP	$VPAC_1$	Ro 25–1553 ($VPAC_2$)	PG 97–269 ($VPAC_1$)
	-VIP (28)	$VPAC_2$		VIP 6–28
	-PHI (27)			
	-PHV (27)			
	-PACAP (27, 38)			

TABLE II
STUDIES OF NEUROPEPTIDES IN CEREBROSPINAL FLUID IN PATIENTS WITH SCHIZOPHRENIA

Peptide	APD therapy	Compared to controls	Associated features
AVP	Drug-free	↔[1, 2]	–
CCK	Drug-free	↓[3–7]	Inversely associated with latency to APD response [6,7]
	Maintained	↓[8]	
CRF	Drug-free	↑	Modestly increased compared to depression[8]
	Drug-free	↔[9–12]	Trend to CRF increase [10]
	Withdrawn	↔[13]	Trend for relapsers to have higher CRF[13]
Dynorphin	Drug-free	↑[14–15]	Associated with severity of symptoms[14] and poor outcome[15]
Endorphin	Drug-free	↓[16]	–
	Drug-free	↓[17–18]	–
	Drug-free	↑[19–23]	Decreased after APD treatment[20–23]
	Drug-free	↔[24–27]	–
Enkephalin	Drug-free	↓[26, 28]	–
Gastrin	Drug-free	↔[29, 30]	–
Hypocretin	Drug free	↔[31]	–
IL-2 and -6	Drug free	↑[32, 33]	–
	Drug-free	↔[34–37]	Trend to IL-6 increase[34]
	Maintained	↔[36]	–
	Withdrawn	↑[38]	Associated with relapse[38]
NT	Drug-free	↓[39–43]	Associated with severity of positive and negative of symptoms[42–44] Associated with latency to APD response [43] Normalization after successful APD treatment[40, 42]
NPY	Drug-free	↔[45, 46]	Increased PYY[46]. Not modifiied by APD treatment[46]
	Drug-free	↑[47]	Associated with duration of illness, brain abnormalities and severity of symptoms[47]
	Withdrawn	↓[47]	–
Oxytocin	Maintained	↔[48]	–
	Withdrawn	↔[48]	–
SOM	Drug-free	↔[5, 10]	Not modified by APD treatment[49]
	Drug-free	↓[49–51]	–
	Drug-free	NA[52, 53, 54]	Reduced by APD treatment
SP	Drug-free	↔[14, 55, 56]	–
TRH	Drug-free	↔[2, 10, 57]	–
VIP	Drug-free	↔[29, 30]	–

AVP, arginine-vasopressin; CCK, cholecystokinin; CRF, corticotropin-releasing factor; IL, interleukin; NPY, neuropeptide Y; NT, neurotensin; SOM, somatostatin; SP, substance P; TRH, thyrotropin-releasing hormone; VIP, vasoactive intestinal peptide; ↑, increase; ↓, decrease; ↔, no changes; APD, antipsychotic drug.

located in the ventral mesencephalon, medial nucleus accumbens (NAcc), septum, hypothalamus, and solitary complex (Vanderhaeghen *et al.*, 1981). Two CCK receptors have been identified, CCK_A (CCK_1) and CCK_B (CCK_2), both G-protein–coupled receptors (GPCRs). The CCK_A receptor has a higher affinity for CCK than gastrin, whereas the CCK_B receptor has similar affinity for CCK and gastrin.

CCK-8 is the predominant form of CCK in the CNS and CSF, and the CNS is the main source of CSF CCK (Hökfelt *et al.*, 1994; Rehfeld and Kruse-Larsen, 1978). CSF CCK concentrations in schizophrenic patients (drug-free and treated with antipsychotic drugs) have been reported to be reduced compared to controls in some studies (Beinfeld and Garver, 1991; Garver *et al.*, 1990; Lotstra *et al.*, 1985; Verbanck *et al.*, 1984) or not different in others (Gerner and Yamada, 1982; Gerner *et al.*, 1985; Rafaelsen and Gjerris, 1985; Tamminga *et al.*, 1986). An association between CSF concentrations of CCK and the latency to antipsychotic drug response has been reported (Beinfeld and Garver, 1991; Garver *et al.*, 1990). See Table II for a summary of the available CSF studies.

The postmortem data suggest disruption of CCK neurotransmission in the temporal lobe, possibly involving the limbic system in schizophrenia (for summary see Table III). CCK-immunoreactivity (IR), CCK mRNA expression, and CCK receptor binding are decreased in the temporal cortex, parahippocampal cortex, hippocampus, and frontal cortex in schizophrenic patients compared to normal controls (Bachus *et al.*, 1997; Farmery *et al.*, 1985; Gabriel *et al.*, 1996; Kerwin *et al.*, 1992; Roberts *et al.*, 1983; Virgo *et al.*, 1995), but discordant results have also been obtained (Bachus *et al.*, 1997; Roberts *et al.*, 1983). Additionally, increased CCK mRNA expression in the substantia nigra of antipsychotic drug-treated schizophrenic patients has been reported (Schalling *et al.*, 1990).

In humans, the gene encoding CCK is located on chromosome 3 (Takahashi *et al.*, 1986). The CCK_A receptor is encoded on chromosome 4, in proximity to the gene encoding the dopamine (DA) D_5 receptor, whereas the CCK_B receptor

–, No positive or negative associations; NA, not assessed.

[1](Sorensen *et al.*, 1985), [2](Gjerris *et al.*, 1985), [3](Lotstra *et al.*, 1985), [4](Verbanck *et al.*, 1984), [5](Gerner and Yamada, 1982), [6](Beinfeld and Garver, 1991), [7](Garver *et al.*, 1990), [8](Banki *et al.*, 1987), [9](Nemeroff *et al.*, 1984), [10](Banki *et al.*, 1992b), [11](Risch *et al.*, 1992), [12](Nishino *et al.*, 1998), [13](Forman *et al.*, 1994), [14](Heikkilä *et al.*, 1990), [15](Lindström, 1996), [16](Zhang *et al.*, 1985), [17](Naber *et al.*, 1981), [18](Pickar *et al.*, 1981), [19](Domschke *et al.*, 1979), [20](Rimon *et al.*, 1980), [21](Lindström *et al.*, 1978), [22](Lindström *et al.*, 1986), [23](Lindström *et al.*, 1992), [24](Emrich *et al.*, 1979), [25](Gerner and Sharp, 1982), [26](Burbach *et al.*, 1979), [27](Nakao *et al.*, 1980), [28](Wen *et al.*, 1983), [29](Gjerris *et al.*, 1984), [30](Rafaelsen and Gjerris, 1985), [31](Nishino *et al.*, 2002), [32](Licinio *et al.*, 1993), [33](Garver *et al.*, 2003), [34](van Kammen *et al.*, 1999), [35](Barak *et al.*, 1995), [36](Katila *et al.*, 1994), [37](el-Mallakh *et al.*, 1993), [38](McAllister *et al.*, 1995), [39](Widerlöv *et al.*, 1982), [40](Lindström *et al.*, 1988), [41](Nemeroff *et al.*, 1989), [42](Breslin *et al.*, 1994), [43](Garver *et al.*, 1991), [44](Sharma *et al.*, 1997), [45](Berrettini *et al.*, 1987), [46](Widerlöv *et al.*, 1988), [47](Peters *et al.*, 1990), [48](Glovinsky *et al.*, 1994), [49](Heikkilä, 1993), [50](Rubinow, 1986), [51](Sharma *et al*, 1995), [52](Bissette *et al.*, 1986), [53](Gerner *et al.*, 1985), [54](Doran *et al.*, 1989), [55](Rimon *et al.*, 1984), [56](Miller *et al.*, 1996), [57](Sharma *et al.*, 2001).

TABLE III

SUMMARY OF RESULTS FROM STUDIES EXAMINING NEUROPEPTIDE SYSTEMS IN POSTMORTEM BRAIN TISSUE FROM SUBJECTS WITH SCHIZOPHRENIA [DATA REPRESENT CHANGES IN IMMUNOREACTIVITY (IR), mRNA EXPRESSION OR BINDING FOR EACH PEPTIDE UNLESS OTHERWISE SPECIFIED]

Peptide	Frontal cortex	Temporal cortex	Entorhinal cortex	Cingulate cortex	Nucleus accumbens	Caudate/ Putamen	Amygdala	Hippocampus	Substantia nigra
AVP	NA	↓IR[1]	NA	NA	NA	NA	NA	NA	NA
CCK	↓ and ↔ mRNA[2,3]	↓mRNA[2]	↓ mRNA[2,3]	↔ mRNA[3]				↓ mRNA[6–8]	
	↓ and ↔ IR[5,6] ↓ binding[4]	↓and ↔ IR[5–8] ↓ binding[4]	↔ IR[9]	↔ IR[8]	↔ IR[5]	↔ IR[5] ↔ binding[4]	↓ and ↔ IR[5,7,8] ↔ binding[4]	↓and ↔ IR[5–8] ↓ binding[4,10]	↑ IR[11]
Dyn	↔ proDyn mRNA[12] ↔κ receptor mRNA[12]	NA	NA	↔ proDyn mRNA[12] ↔κ receptor mRNA[12]	NA	NA	↔ proDyn mRNA[13]	NA	↔ Dyn-IR[14]
Enk	↑ IR[15]	NA	NA	NA		↓ IR[5] ↔ proEnk mRNA[16]	↔ IR[17]	NA	↑ IR[15]
CRF	↔ IR[6]	↔ IR[6]	NA	↓ IR[6]	NA	NA	NA	NA	NA
Gal	↔ IR[18]	↓and ↔ IR[1,18]	NA	NA	NA	NA	NA	NA	NA
NT	↑ IR[19] ↓ binding[20]	↔ IR[8]	↓ binding[21, 22]	↔ IR[8] ↓ binding[20]	↔ IR[5,8]	↔ IR[8] ↓ binding[20,23]	↔ IR[8,17]	↔ IR[8]	↑ binding[24]

NPY	Altered distribution[25] ↓ and ↔ mRNA[26,27] ↔ NPY_1 mRNA[28] ↔ NPY_2 mRNA[28]	↓ IR[1,6] ↓ PYY-IR[1]	NA	↓ IR[6]	NA	NA	↔ IR[17,29]	Altered morphology in NPY fibers in CA4[30]	NA
SOM	↓ IR[6,19]	↓ IR[6]	NA	↓ IR[6]	NA	↔ IR[8]	↔ IR[8,29]	↓ IR[6–8]	NA
SP	↔ mRNA[31] ↑ and ↔ IR[5,15] ↑ NK_1-IR[31]	↔ mRNA[32] ↑ IR[15] ↔ binding[32]	NA	↔ IR[8]	↔ IR[5,15]	↔ mRNA[16] ↔ IR[5,15]	↓ mRNA[32] ↔ IR[8] ↔ binding[32]	↑ IR[8,15]	↑ IR[15] ↔ IR[14,15]
TRH	↓ IR[19]	NA	NA	NA	↔ IR[19]	↔ IR[19]	↔ IR[19]	NA	NA
VIP	↔ IR[6,8]	↔ IR[6,8]	↔ IR[9]	↔ IR[6]	NA	↔ IR[8]	↑ IR[8,17]	↔ IR[6, 8]	NA

AVP, arginine-vasopressin; CCK, cholecystokinin; CRF, corticotropin-releasing factor; Dyn, dynorphin; Enk, enkephalin; Gal, galanin; NPY, neuropeptide Y; NT, neurotensin; PYY, peptide YY; SOM, somatostatin; SP, substance P; TRH, thyrotropin-releasing hormone; VIP, vasoactive intestinal peptide; ↑, increased; ↓, decreased; ↔, no change; NA, not assessed; IR: immunoreactivity.

[1](Frederiksen *et al.*, 1991), [2](Virgo *et al.*, 1995), [3](Bachus *et al.*, 1997), [4](Farmery *et al.*, 1985), [5](Kleinman *et al.*, 1985), [6](Gabriel *et al.*, 1996), [7](Ferrier *et al.*, 1983), [8](Roberts *et al.*, 1983), [9](Perry *et al.*, 1981), [10](Kerwin *et al.*, 1992), [11](Schalling *et al.*, 1990), [12](Peckys and Hurd, 2001), [13](Hurd, 2002), [14](Iadarola *et al.*, 1991), [15](Toru *et al.*, 1988), [16](Harrington *et al.*, 1995), [17](Zech *et al.*, 1986), [18](Gabriel *et al.*, 1994), [19](Nemeroff *et al.*, 1983), [20](Lahti *et al.*, 1998), [21](Hamid *et al.*, 2002), [22](Wolf *et al.*, 1995), [23](Palacios *et al.*, 1991), [24](Uhl and Kuhar, 1984), [25](Ikeda *et al.*, 2004), [26](Kuromitsu *et al.*, 2001), [27](Caberlotto and Hurd, 1999), [28](Caberlotto and Hurd, 2001), [29](Beal *et al.*, 1987), [30](Iritani *et al.*, 2000), [31](Tooney *et al.*, 2001), [32](Carletti *et al.*, 2005).

is encoded on chromosome 11, in proximity to the gene encoding the DA D_4 receptor (Huppi *et al.*, 1995). On the basis of the similarity in expression patterns, biological effects, and their proximity in the human genome, it has been proposed that receptors for CCK and DA may possibly interact with one another, perhaps via coregulation (Huppi *et al.*, 1995). Two studies of polymorphisms in the CCK gene found no association with schizophrenia (Bowen *et al.*, 1998; Hattori *et al.*, 2001a). In contrast, a significant association was found between a polymorphism in the promoter region of the CCK gene and schizophrenia in a family-based analysis (Wang *et al.*, 2002). Polymorphisms in the CCK_A receptor gene and its promoter have also been associated with schizophrenia (Tachikawa *et al.*, 2000, 2001), specifically with the paranoid type of schizophrenia (Tachikawa *et al.*, 2000, 2001) and positive symptom severity (Lu *et al.*, 2004; Zhang *et al.*, 2000). Two studies have failed to find any association between polymorphisms in the CCK_B receptor gene and schizophrenia (Hattori *et al.*, 2001b; Tachikawa *et al.*, 1999).

On the basis of biochemical and behavioral data demonstrating antidopaminergic and antipsychotic-like effects of central CCK administration (for review see Nair *et al.*, 1986), the efficacies of CCK-8, CCK-33, and the decapeptide caerulein (a mixed CCK_A and CCK_B receptor agonist) have been tested in schizophrenic patients in more than 20 clinical trials (for a summary see Table IV). Most of these trials involved i.v. administration of CCK or caerulein (single to ten doses), alone or in combination with antipsychotic drug maintenance therapy, to chronic schizophrenic patients resistant to neuroleptic treatment. Initial open or single-blind studies reported promising findings of symptom relief that lasted up to several weeks in at least a subset of patients (for review see Montgomery and Green, 1988 and Nair *et al.*, 1986). However, 7 out of 10 double-blind studies reported no difference from placebo (Albus *et al.*, 1986; Hommer *et al.*, 1984; Itoh *et al.*, 1986; Lotstra *et al.*, 1984, 1985; Mattes *et al.*, 1985; Nair *et al.*, 1984, 1985; Peselow *et al.*, 1987; Tamminga *et al.*, 1986; Verhoeven *et al.*, 1986). Moreover, caerulein monotherapy was found to be ineffective in two small clinical trials in schizophrenia (Lotstra *et al.*, 1984; Tamminga *et al.*, 1986). The last clinical trial with a CCK receptor agonist was nearly 20 years ago. The lack of efficacy of CCK in these trials could be related to the small number of patients, inadequate dosing, poor brain penetration, or insufficient treatment duration. The possibility that CCK receptor agonists may represent a novel treatment option for a subset of patients with schizophrenia probably merits additional study.

On the basis of the observation that CCK and CCK analogs stimulate midbrain DA cell firing (Skirboll *et al.*, 1981), the antipsychotic potential of the CCK receptor antagonist proglumide was examined. Three clinical trials failed to demonstrate efficacy of proglumide in the treatment of schizophrenia (Hicks *et al.*, 1989; Innis *et al.*, 1986; Whiteford *et al.*, 1992).

The few clinical studies evaluating gastrin found no evidence of a role for this peptide in the pathophysiology of schizophrenia (Detera-Wadleigh *et al.*, 1987; Gjerris *et al.*, 1984; Rafaelsen and Gjerris, 1985).

III. Corticotropin-Releasing Factor

The importance of stress on the course of schizophrenia is well established. Stressful life events are associated with the onset of schizophrenia (Gruen and Baron, 1984; Leff and Vaughn, 1980; Lukoff *et al.*, 1984), relapse frequency, and psychotic decompensation (Altamura *et al.*, 1999; Gispen-de Wied, 2000; Howes *et al.*, 2004; Norman *et al.*, 2002). Additionally, schizophrenic patients are more vulnerable to stress associated with minor life events (Cotter and Pariante, 2002; Gispen-de Wied, 2000). The 41 amino acid peptide CRF coordinates the mammalian stress response in part via regulation of the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system (ANS). In the CNS, CRF-containing neurons are found in the hypothalamus, amygdala, cerebral cortex, septum, bed nucleus of the stria terminalis (BNST), cerebellum, brain stem, and spinal cord (Fischman and Moldow, 1982). Following exposure to stress, CRF is released from nerve terminals in the paraventricular nucleus of the hypothalamus (PVN) into the portal circulation, binds to CRF receptors located in the anterior pituitary, and induces the release of adrenocorticotropic hormone (ACTH) into the general circulation. ACTH stimulates the release of cortisol from the adrenal cortex. Cortisol is the final mediator of the stress response, modulating glucose metabolism, blood pressure, and immune function. Finally, cortisol feeds back negatively on the HPA axis, binding to glucocorticoid receptors at the level of the hypothalamus, hippocampus, and anterior pituitary. Other members of the CRF family including the urocortins and perhaps urotensin and savagine also participate in regulation of the stress response (Skelton *et al.*, 2000). CRF has two known receptors, CRF_1 and CRF_2 (also known as the urocortin receptor). Although still under debate, anxiogenic and anxiolytic effects are attributed to activation of CRF_1 and CRF_2 receptors, respectively (Nemeroff and Vale, 2005).

Some, but not all studies report a modest increase in CSF CRF concentrations in schizophrenia, although of lesser magnitude than those found in depression (Banki *et al.*, 1987, 1992a; Nishino *et al.*, 1998). Further, discontinuation of haloperidol maintenance therapy in chronic stable schizophrenic patients is associated with increased CSF CRF concentrations (Forman *et al.*, 1994). In postmortem tissue, CRF-IR is decreased in the cingulate gyrus but not in the frontal, temporal, or occipital cortices of schizophrenic patients with cognitive impairment (Frederiksen *et al.*, 1991; Gabriel *et al.*, 1996). To the best of our

TABLE IV

THE RESULTS OF CLINICAL TRIALS WITH NEUROPEPTIDE RECEPTOR AGONISTS AND ANTAGONISTS IN SCHIZOPHRENIC PATIENTS

Compound	Class	Drug administration	Study design	Schizophrenic patient population	APD therapy	Outcome	References
CCK-33	CCK_A, CCK_B agonist	Single	Open	6 Chronic paranoid	Maintained	Improvement	Nair *et al.* (1982)
		Single	Open	21 Chronic resistant	Maintained	Improvement	Nair *et al.* (1983)
CCK-8	CCK_A, CCK_B agonist	Single	Open	8 Chronic resistant	Maintained	Improvement	Bloom *et al.* (1983)
		4 d, b.i.d.	Double-blind cross-over	4 Chronic resistant	Maintained	Not different from placebo	Hommer *et al.* (1984)
		1/wk for 8 wk	Double-blind w/placebo	9 Chronic	Maintained	Improvement in cognition and delusions	Nair *et al.* (1984)
		1/wk for 8 wk	Double-blind w/placebo	14 Chronic	Maintained	Improvement	Nair *et al.* (1985)
		Single	Double-blind cross-over	30 Chronic resistant	Maintained	Not different from placebo	Peselow *et al.* (1987)
Caerulein	CCK_A, CCK_B agonist	Single	Open	20 Chronic	Maintained	Improvement	Moroji *et al.* (1982)
		1–2 wk, q.d.	Open	58 Chronic resistant	Maintained	Improvement in 23/58 cases	Itoh *et al.* (1982)
		2 wk, 1/wk	Open	6 Chronic	Maintained	Improvement	Albus *et al.* (1984)
		3 wk, 1/wk	Double-blind w/placebo	10 Chronic	Maintained	Not different from placebo	Albus *et al.* (1984)
		Single	Double-blind cross-over	9 Chronic	Drug-free	Not different from placebo	Lotstra *et al.* (1984)
		4 wk, b.i.d.	Double-blind cross-over	8 Chronic resistant	Maintained	Not different from placebo	Hommer *et al.* (1984)
		1/wk for 2 wk	Single blind	6 Chronic	Maintained	Improvement	van Ree *et al.* (1984)

		Single	Double-blind cross-over	17 Chronic	Maintained	Not different from placebo	Mattes *et al.* (1985)
		Single	Open	9 Chronic resistant	Maintained	No improvement	Boza and Rotondo (1985)
		5 d, q.d.	Double-blind cross-over	5 Chronic	Drug-free	Not different from placebo	Tamminga *et al.* (1986)
		7 d, q.d.	Double-blind w/placebo	135 Chronic	Maintained	Not different from placebo	Itoh *et al.* (1986)
		1 wk, 3 wk	Double-blind w/placebo	10 Chronic	Maintained	Not different from placebo	Albus *et al.* (1986)
		7× in 3 wk	Double-blind w/placebo	15 Chronic	Maintained	Improvement, especially in patients with less negative symptoms	Verhoeven *et al.* (1986)
Proglumide	CCK_A, CCK_B	4–7 d, q.d.	Double-blind cross-over	4 Chronic	Maintained	No improvement	Innis *et al.* (1986)
	antagonist	4 wk, q.d.	Open	4 Chronic resistant	Maintained	No improvement	Hicks *et al.* (1989)
		4 wk, b.i.d.	Single-blind	7 Chronic resistant	Maintained	No improvement, worsening in some cases	Hicks *et al.* (1989)
		4 wk, q.d.	Double-blind w/placebo	14 Chronic resistant	Maintained	Not different from placebo	Whiteford *et al.* (1992)
DTγE	μ, κ Agonist	7 d, q.d.	Open	6 Chronic resistant	Drug-free	Improvement of psychotic symptoms	Verhoeven *et al.* (1979)
		8 d, q.d.	Double-blind cross-over	8 Chronic resistant	Maintained (6)	Improvement of psychotic symptoms	Verhoeven *et al.* (1979)
		4 d, q.d.	Double-blind cross-over	13 Chronic resistant	Maintained	Not different from placebo	Emrich *et al.* (1980)
		3 d, q.d.	Open	10 Chronic	Maintained	No improvement	Casey *et al.* (1981)
		10 d, q.d.	Open	11 Chronic	Maintained	No improvement, euphoria in 3 cases	Manchanda and Hirsch (1981)
		10 d, q.d.	Double-blind w/placebo	17 Chronic	Maintained	Improvement	Verhoeven *et al.* (1982)

(*Continued*)

TABLE IV (*Continued*)

THE RESULTS OF CLINICAL TRIALS WITH NEUROPEPTIDE RECEPTOR AGONISTS AND ANTAGONISTS IN SCHIZOPHRENIC PATIENTS

Compound	Class	Drug administration	Study design	Schizophrenic patient population	APD therapy	Outcome	References
		12 d, q.d.	Open	8 Mixed	Drug-free	Improvement in 6	Meltzer *et al.* (1982)
		Single	Open	5 Chronic	Drug-free	No improvement	Tamminga *et al.* (1981)
		Single	Open	4 Chronic	Maintained	No improvement	Korsgaard *et al.* (1982)
		1/d, 8 d	Double-blind cross-over	9 Chronic	Maintained	Transient but modest improvement	Volavka *et al.* (1983)
		14 d, q.d.	Open	6 Chronic	Maintained	No improvement	Mizuki *et al.* (1986)
DEγE	μ, κ Agonist	10 d, q.d.	Double-blind cross-over	18 Chronic	Drug-free	Improvement in subset (hebephrenic, paranoid types with less negative symptoms)	Verhoeven *et al.* (1984b)
		7× in 3wk	Double-blind w/placebo	15 Chronic	Maintained	Improvement in subset (less negative symptoms)	Verhoeven *et al.* (1986)
		4 wk, q.d.	Double-blind var. doses	93 Chronic	Maintained	Not different from placebo	Azorin *et al.* (1990)
		4 wk, q.d.	Double-blind w/placebo	31 Chronic	Drug-free	Not different from placebo	Montgomery *et al.* (1992)
Naltrexone	μ, κ, δ Antagonist	6 wk, q.d. to t.i.d.	Open	5 Chronic	Maintained	No improvement	Mielke and Gallant (1977)
		7 wk, q.d.	Double-blind cross-over	8 Chronic	Drug-free	Not different from placebo	Gitlin *et al.* (1981)
		14 wk, b.i.d.	Double-blind w/placebo	4 Chronic negative	Maintained	Not different from placebo	Marchesi *et al.* (1992)
		2 wk, b.i.d.	Double-blind w/placebo	9 Chronic	Maintained	Improvement in positive and negative symptoms	Marchesi *et al.* (1995)

Naloxone	μ, κ, δ Antagonist	Single	Single blind	6 Chronic	Maintained	Decreased hallucinations in 4 patients	Gunne *et al.* (1977)
		1 d, b.i.d.	Double-blind w/placebo	12 Chronic	Maintained	Not different from placebo	Kurland *et al.* (1977)
		2 d, q.d.	Double-blind cross-over	9 Chronic	Maintained	Decreased hallucinations	Watson *et al.* (1978)
		Single	Double-blind cross-over	9 Chronic	Maintained	No improvement	Lipinski *et al.* (1979)
		2 d, q.d.	Double-blind cross-over	14 Chronic	Maintained	Decreased hallucinations	Berger *et al.* (1981)
		2 d, q.d.	Double-blind cross-over	11 Chronic	Drug free	Not different from placebo	Sethi and Prakash (1981)
		Single	Double-blind cross-over	13 Chronic hallucinating	Maintained		Freeman and Fairburn, (1981)
		2 d, q.d.	Double-blind cross-over	32 Chronic	Drug free (13) Maintained (19)	Drug-free: not different from placebo APD-treated: improvement particularly in hallucinations	Pickar *et al.* (1982)
		2 d, q.d.	Double-blind cross-over	4 Chronic	Maintained	Improvement in noncatatonics	Cohen *et al.* (1985)
		4 d, q.d.	Double-blind cross-over	6 Chronic	Maintained	Not different from placebo	Naber and Leibl (1983)
		5 d, q.d.	Double-blind cross-over	12 Chronic	Maintained	Not different from placebo	Naber *et al.* (1983)
		4 d, q.d.	Double-blind cross-over	43 Chronic	Maintained	Not different from placebo	Pickar *et al.* (1989)
		4 d, q.d.	Double-blind cross-over	10	Maintained	Placebo showed a slightly better effect	Verhoeven *et al.* (1984a)
Interleukin-2		Single	Open	44 Nonpsychiatric	Drug free	Induced psychotic symptoms	Denicoff *et al.* (1987)

(*Continued*)

TABLE IV (*Continued*)
THE RESULTS OF CLINICAL TRIALS WITH NEUROPEPTIDE RECEPTOR AGONISTS AND ANTAGONISTS IN SCHIZOPHRENIC PATIENTS

Compound	Class	Drug administration	Study design	Schizophrenic patient population	APD therapy	Outcome	References
Oxytocin		6–10 d, q.d.	Open	Number not reported	Not reported	Improvement, particularly in acute cases	Bujanow (1972); Bujanow (1974)
SR 48692	Neurotensin receptor (NT_1) antagonist	6 wk, q.d.	Double-blind multi-arm	63 Schizophrenics and schizoaffective	Drug-free	Not different from placebo	Meltzer *et al.* (2004)
Secretin		Single	Double-blind w/placebo	11 Severely ill, resistant	Maintained	Not different from placebo, improvement in a subgroup	Sheitman *et al.* (2004)
Osanetant	NK_3 antagonist	6 wk, q.d.	Double-blind multi-arm	71 Schizophrenics and schizoaffective	Drug-free	Improvement in positive and cognitive symptoms, Very mild side effects	Meltzer *et al.*, 2004)
TRH		1/wk for 2 wk	Open	10 Chronic	Drug-free	Improvement in affect and thought	Wilson *et al.* (1973)
		5 d, q.d.	Double-blind cross-over	3 Chronic resistant	Drug-free	Worsening of symptoms in 2 cases	Bigelow *et al.* (1975)
		14 d, t.i.d.	Open	9 Chronic	Drug-free	Worsening of symptoms	Davis *et al.* (1975)
		3 wk, q.d.	Double-blind cross-over	5 Chronic	Maintained	Not different from placebo	Clark *et al.* (1975)

			4 d, q.d.	Double-blind cross-over	10 Chronic	Drug-free	Not different from placebo, Increase in TSH	Lindström *et al.* (1977)
			14 d, q.d.	Double-blind w/placebo	70 Chronic	Maintained	Global improvement, onset within a week	Inanaga *et al.* (1978)
			Single	Single blind	5 Chronic	Drug-free	Improvement	Prange *et al.* (1979)
			Single	Double-blind w/placebo	12 Chronic	Drug-free	Transient improvement in psychotic symptoms	Prange *et al.* (1979)
			14 d, q.d.	Double-blind cross-over	11 Chronic	Maintained	Not different from placebo, improvement in affect in some cases	Kobayashi *et al.* (1980)
			15× in 30 d	Open	10 Chronic	Maintained	Improvement of negative symptoms, Transient borderline hyperthyroidism	Brambilla *et al.* (1986)
	DN-1417	TRH analogue	14 d, q.d.	Open	6 Chronic	Maintained	Improvement in hallucinations and thought content, Increase in frontal EEG activity	Mizuki *et al.* (1986)

CCK, cholecystokinin; DTγE, des-tyrosine-gamma-endorphin; DEγE, des-enkephalin-gamma-endorphin; TRH, thyrotropin-releasing hormone; APD, antipsychotic drug; wk, week; d, day; q.d., once daily; b.i.d., twice daily; t.i.d., three times a day.

knowledge, association studies between CRF system genes and schizophrenia or the clinical efficacy of CRF-related compounds in schizophrenia have not been examined.

Several groups have reported increased baseline plasma cortisol concentrations in schizophrenic patients, although of a lesser magnitude than in depressed patients (for review see Altamura *et al.*, 1999; Gispen-de Wied, 2000; Lieberman and Koreen, 1993). This hypercortisolemia has been postulated to be associated with an increase in inflammatory cytokines (Altamura *et al.*, 1999). In addition, a subset of schizophrenic patients displays cortisol hyposecretion following pharmacological challenge of the HPA axis (i.e., dexamethasone, apomorphine, and DA receptor antagonists) (Altamura *et al.*, 1989; Coryell and Tsuang, 1992; McGauley *et al.*, 1989; Meltzer *et al.*, 2001; Tandon *et al.*, 2000, 1991; Yeragani, 1990). In the dexamethasone suppression test (DST), the best studied test of HPA axis function in schizophrenia, rates of nonsuppression vary from 0% to 73%, and are higher in drug-free than in medicated patients (Tandon *et al.*, 1991). DST nonsuppression, particularly in the drug-free state, is associated with negative symptoms and is a predictor of poor outcome (Altamura *et al.*, 1999; Coryell and Tsuang, 1992; Tandon *et al.*, 1991, 2000). An abnormal stress response in schizophrenia is further supported by alterations in the regulation of the HPA axis and the ANS. Alterations of ANS function in schizophrenia include increased basal heart rate and abnormalities in temperature regulation and skin conductance (Gispen-de Wied, 2000).

IV. Interleukins

Cytokines are small peptides produced by immune cells that serve as an important component of the immune response. The effects of cytokines on the CNS have received considerable attention. Cytokines have both direct and indirect access to the brain via the so called "leaky" regions in the blood-brain barrier (i.e., the circumventricular organs), via cytokine-specific transporters in endothelial cells and via vagal afferents. Moreover, interleukin 2 (IL-2) and its receptor are widely distributed in the brain (Lapchak *et al.*, 1991). Of particular interest to schizophrenia are studies demonstrating cytokine regulation of the mesolimbic DA system. IL-2 enhances DA release in striatal slice preparations (Lapchak, 1992; Petitto *et al.*, 1997) and *in vivo*, systemic IL-2 administration enhances norepinephrine (NE) and DA turnover in the hypothalamus and prefrontal cortex, respectively (Zalcman *et al.*, 1994) and induces hyperlocomotion (Petitto *et al.*, 1997). Further support for a possible role of cytokines in the pathogenesis of schizophrenia was provided by the demonstration that prenatal lipopolysaccaride exposure produces disruptions in sensorimotor gating (one of

the core features of schizophrenia), increased serum IL-2 and IL-6 concentrations, and distinct abnormalities in the DA system and glial cells in mesolimbic regions in adult rats. The effect of prenatal lipopolysaccaride exposure on sensorimotor gating was reversed by antipsychotic drug administration (Borrell *et al.*, 2002).

Several additional lines of evidence suggest that cytokines may be involved in the pathogenesis of schizophrenia. First, IL-2 administration produced psychotic-like symptoms (paranoia and perceptual abnormalities) in nonpsychiatric patients (Denicoff *et al.*, 1987). Second, epidemiological studies have repeatedly demonstrated associations between pre- and perinatal (see Pearce, 2001 for review) as well as childhood (Koponen *et al.*, 2004) viral infections and schizophrenia. Moreover, several studies have reported immune abnormalities in schizophrenia (reviewed in Muller *et al.*, 2000; Rothermundt *et al.*, 1998). These abnormalities range from alterations in white blood cell counts and activation, to changes in serum and CSF cytokine concentrations, and cytokine production by activated lymphocytes. The most consistent cytokine findings in schizophrenia are decreased mitogen stimulated IL-2 and IFN-γ production by CD4 lymphocytes, increased CSF and serum concentrations of IL-2 and IL-6, and increased serum soluble IL-2 receptors (for review see Smith, 1992). It has been suggested that abnormally activated T lymphocytes oversecrete IL-2 leading to both increased serum IL-2 concentrations and depletion of IL-2 in lymphocytes themselves (Smith, 1992; Smith and Maes, 1995). These abnormalities are more pronounced in patients with treatment-resistant schizophrenia, and have been associated with the severity of positive symptoms and poor treatment outcome (Arolt *et al.*, 2000; McAllister *et al.*, 1995; Zhang *et al.*, 2002, 2005). In addition, antipsychotic drugs seem to have an inhibitory effect on inflammatory cytokines (Arolt *et al.*, 2000; Cazzullo *et al.*, 2002; Rothermundt *et al.*, 2000; Sirota *et al.*, 2005; Song *et al.*, 2000; Zhang *et al.*, 2005 but see also Kim *et al.*, 1995; Muller *et al.*, 1997). In addition to the variability commonly observed in clinical studies, cytokine studies exhibit differences in bioassays, cell preparation techniques, ongoing infectious diseases, production of counterregulatory cytokines, stress, and circadian variation. It has been suggested that abnormally activated T lymphocytes oversecrete IL-2 leading to both increased serum IL-2 concentrations and depletion of IL-2 in lymphocytes themselves (Smith, 1992; Smith and Maes, 1995).

A study with 230 schizophrenic patients found an association between a single nucleotide polymorphism in the IL-2 gene and schizophrenia (Schwarz *et al.*, 2006). It is interesting to note that a double-blind, placebo-controlled study revealed that addition of the cyclo-oxygenase 2 inhibitor celocoxib (an anti-inflammatory drug used in the treatment of rheumatoid arthritis) to risperidone markedly improved psychotic symptoms in schizophrenic patients (Muller *et al.*, 2004). Many of these immunologic abnormalities in schizophrenia resemble the natural history (including age of onset, relapsing course, and differential gender

distribution) of a number of well-known immune diseases such as rheumatoid arthritis and systemic lupus erythematosus. Additionally, proinflammatory cytokine concentrations are increased by stress and have been postulated to play a key role in depression (Raison *et al.*, 2006). Overall, it is tempting to posit a role for proinflammatory cytokines, particularly IL-2, in the pathogenesis of schizophrenia, mostly in mediating the increase in schizophrenia associated with birth season and perinatal insult.

V. Neurotensin

The 13 amino acid-containing peptide NT was first isolated by Carraway and Leeman 1973 and is encoded on human chromosome 12 (Marondel *et al.*, 1996). NT is widely distributed in the gastrointestinal tract, circulatory system, and in the central and peripheral nervous systems. In the CNS, NT is involved in regulation of reward, pain and body temperature, and has been hypothesized to play a role in the pathophysiology of schizophrenia, in the mechanism of action of antipsychotic drugs and in drug abuse. There are three identified NT receptors (NT_1–NT_3) and a fourth putative receptor (NT_4). NT_1 and NT_2 are GPCRs, whereas the NT_3 and putative NT_4 receptors are members of the vacuolar sorting and LDL receptor families (Dobner, 2005; Vincent *et al.*, 1999).

NT was first proposed by our group to be an endogenous antipsychotic in 1980 based on the close anatomical and neurochemical interactions between NT and the DA system and the striking behavioral similarities between the effects of antipsychotic drugs and centrally administered NT (Nemeroff, 1980). Since then, copious data supporting this hypothesis have been published (for review see Cáceda *et al.*, 2003; Dobner, 2005; Kinkead and Nemeroff, 2004).

The strongest clinical research evidence for the involvement of NT in schizophrenia has been provided by the measurement of NT concentrations in the CSF of drug-free and antipsychotic drug-treated schizophrenic patients. CSF NT concentrations are independent of serum concentrations and are believed to largely reflect CNS NTergic activity (Widerlöv *et al.*, 1982). CSF NT concentrations did not vary with patient age, duration of disease, or previous antipsychotic drug treatment (Lindström *et al.*, 1988). Low CSF NT concentrations have consistently been found in a subset of drug-free schizophrenic patients relative to normal volunteers and patients with other psychiatric disorders (Breslin *et al.*, 1994; Lindström *et al.*, 1988; Manberg *et al.*, 1985; Nemeroff *et al.*, 1989; Sharma *et al.*, 1994; Widerlöv *et al.*, 1982). In this subset of patients, clinical improvement (especially in negative symptoms) was associated with normalization of CSF NT concentrations (Breslin *et al.*, 1994; Garver *et al.*, 1991; Sharma *et al.*, 1997).

Low CSF NT concentrations were positively correlated with the severity of psychopathology, including thought disorder, deficit symptoms, disorganized behavior, and impaired functioning (Garver *et al.*, 1991; Sharma *et al.*, 1997). There is considerable specificity to schizophrenic patients, as CSF NT concentrations were unchanged in patients with other neuropsychiatric conditions, including anorexia/bulimia, depression, premenstrual syndrome, and Huntington's disease when compared to age-matched control subjects (Nemeroff *et al.*, 1989). Until technological advances to assess CNS NTergic neurotransmission such as the use of PET or SPECT to measure NT receptor subtype ligands are available, CSF studies represent the best *in vivo* evidence of NT dysfunction in schizophrenia. Taken together, these studies reveal a subset of schizophrenic patients with reduced NT neurotransmission.

Examination of the NT system in human postmortem tissue studies has generated variable results with several negative studies (Manberg *et al.*, 1982; Palacios *et al.*, 1991; Zech *et al.*, 1986). Among the positive findings are increased NT-IR in the frontal cortex (Manberg *et al.*, 1985; Nemeroff *et al.*, 1983), decreased NT receptor binding in layer II of the entorhinal cortex, caudate/putamen, and cingulate cortex (Hamid *et al.*, 2002; Lahti *et al.*, 1998; Wolf *et al.*, 1995), and increased NT receptor binding in the substantia nigra of medicated patients (Uhl and Kuhar, 1984). See Table III for summary of results.

Several polymorphisms have been identified in the noncoding region of the human NT_1 receptor gene (Austin *et al.*, 2000a; Huezo-Diaz *et al.*, 2004; Le *et al.*, 1997a,b; Watson *et al.*, 1993). However, no associations have been found between genetic variations in the NT or NT_1 receptor genes and schizophrenia (Austin *et al.*, 2000a,b; Huezo-Diaz *et al.*, 2004).

In contrast to the hypothesis that NT serves as an endogenous antipsychotic, is evidence that NT receptor antagonists may exhibit antipsychotic properties. Chronic administration of an NT receptor antagonist, like antipsychotic drugs, produces depolarization block in the ventral tegmental area (Santucci *et al.*, 1997). Additionally, similar to the effects of both NT receptor agonists (Feifel *et al.*, 1999) and antipsychotic drugs (Bakshi and Geyer, 1995; Geyer *et al.*, 2001), acute administration of an NT receptor antagonist prevents amphetamine- and dizocilpine-induced disruption of prepulse inhibition (PPI) of the startle response in rats (Cáceda *et al.*, submitted for publication). These seemingly contradictory findings are most likely explained by the different roles played by distinct anatomical NT circuits. NT has antipsychotic-like behavioral effects in the NAcc but stimulant-like behavioral effects in the VTA (Cáceda *et al.*, 2005; Feifel *et al.*, 1997; Kalivas *et al.*, 1981, 1984). The antipsychotic-like effects of systemic NT receptor antagonists may be due to blockade of NT neurotransmission in regions other than the NAcc, such as the VTA or subiculum. Despite this promissory preclinical evidence that NT receptor antagonists may have antipsychotic drug

properties, an NT receptor antagonist was ineffective in the treatment of refractory schizophrenic patients (Meltzer *et al.*, 2004). The patient population used in this study (refractory to antipsychotic drug treatment) and the testing of only a single (possibly suboptimal) dose of the NT receptor antagonist limits any firm conclusions to be drawn. Although NT receptor agonists have repeatedly been shown to possess antipsychotic-like behavioral effects in laboratory animals, no clinical trials using an NT receptor agonist have been conducted. This delay is due to the lack of a specific and potent small molecule NT receptor agonist.

VI. Neuropeptide Y

The pancreatic polypeptide family is composed of NPY, pancreatic polypeptide, peptide YY (PYY), and polypeptide Y. NPY is 36 amino acids long and is encoded on human chromosome 7. The five known receptors for NPY (NPY_1–NPY_5) are all GPCRs. Central NPY systems have been implicated in anxiety, major depression, bipolar disorder, suicide, and schizophrenia (for review see Obuchowicz *et al.*, 2004).

Of the three studies in which NPY concentrations were measured in the CSF of schizophrenic patients (Berrettini *et al.*, 1987; Peters *et al.*, 1990; Widerlöv *et al.*, 1988), only one found an increase in schizophrenic patients (drug free and after haloperidol withdrawal) compared to normal controls (Peters *et al.*, 1990). In this study, CSF concentrations of NPY positively correlated with duration of illness, the presence of abnormalities on brain CT scans and severity of clinical symptomatology in stable patients (Peters *et al.*, 1990).

Abnormal distribution of NPY positive interneurons (Ikeda *et al.*, 2004) and decreased NPY mRNA expression (Kuromitsu *et al.*, 2001) were observed in the dorsal prefrontal cortex of schizophrenic patients in postmortem studies, especially in the disorganized and paranoid type (but see Caberlotto and Hurd, 1999, 2001). Additionally, decreased NPY-IR was reported in the cingulate and temporal cortices of schizophrenic patients (Frederiksen *et al.*, 1991; Gabriel *et al.*, 1996). Finally, morphological alterations in NPY positive fibers have been reported in the CA4 region of the hippocampus in schizophrenia (Iritani *et al.*, 2000; Table III).

An association between a polymorphism in the promoter region of NPY and schizophrenia was reported (Buckland *et al.*, 2004; Itokawa *et al.*, 2003) but not replicated (Duan *et al.*, 2005; Lindberg *et al.*, 2006). In addition, two studies found no association between polymorphisms in the NPY gene and schizophrenia (Detera-Wadleigh *et al.*, 1987; Duan *et al.*, 2005). There are no published clinical trials in schizophrenia with compounds that increase or decrease NPY neurotransmission.

VII. Opioid Peptides

Three families of opioid peptides are known, each arising from different genes and precursor molecules: enkephalin A, proopiomelanocortin (POMC), and proenkephalin B or prodynorphin. Met-enkephalin, β-endorphin, and dynorphin are the best known molecules (Table I). The precursor molecules undergo extensive posttranslational modifications in the Golgi apparatus, including cleavage and acetylation. Opioid peptides interact with the μ, κ, σ, and δ GPCRs. The opioid receptors are associated with, and display differential selectivity for, each of the opioid peptides.

A. Endorphins

Endorphins, relatively selective for the μ receptor, are widely expressed in the brain and spinal cord, particularly in the median eminence, periaqueductal gray matter, and substantia nigra (Zamir *et al.*, 1984). A large body of research has investigated whether endorphins play a preeminent role in pain regulation, reward, and drug addiction, as well as in schizophrenia and mood disorders. The hypothesized role of endorphins in schizophrenia is based on the antipsychotic-like effects of γ-endorphin in rodents and the reports of elevated concentrations of nonbiologically active α and γ-endorphins in the hypothalamus of schizophrenic patients (Wiegant *et al.*, 1988). However, the existent clinical research data do not support a role for endorphins in the pathophysiology of schizophrenia (for review see De Wied and Sigling, 2002; Wiegant *et al.*, 1992). CSF β-endorphin concentrations in unmedicated schizophrenic patients have been reported to be decreased (Naber *et al.*, 1981; Pickar *et al.*, 1981), unchanged (Burbach *et al.*, 1979; Emrich *et al.*, 1979; Gerner and Sharp, 1982), or increased (Domschke *et al.*, 1979; Lindström *et al.*, 1986, 1992) and decreased after antipsychotic drug treatment (Lindström *et al.*, 1986, 1992; Rimon *et al.*, 1980).

Initial open and double-blind studies with β-endorphin or [Des-Tyr1]-gamma-endorphin (DTγE) alone or in combination with antipsychotic drugs in schizophrenic patients produced some positive results (see Wiegant *et al.*, 1992 for review), especially in the hebephrenic and paranoid subtypes (Verhoeven *et al.*, 1979, 1984). However, double-blind, placebo-controlled studies with more than 90 patients failed to demonstrate efficacy compared to placebo (Azorin *et al.*, 1990; Montgomery *et al.*, 1992). Similarly, although case reports and small pilot studies reported that the opiate antagonists, naltrexone and naloxone, demonstrate clinical efficacy (particularly against hallucinations) double-blind placebo-controlled studies failed to replicate these findings (see Welch and Thompson, 1994 for review).

B. Dynorphin

κ Opiate receptors have a high affinity and are relatively selective for dynorphin. Dynorphin is found in high concentrations in the neo- and allocortices, caudate/putamen, NAcc, amygdala, BNST, hypothalamus, medial pretectal area, nucleus of the optic tract, periaqueductal gray, raphe nuclei, and in brain stem nuclei involved in pain and nociception (Fallon and Leslie, 1986). Increased CSF concentrations of dynorphin were reported in unmedicated schizophrenic patients compared to healthy controls or other psychiatric patients (Heikkilä *et al.*, 1990; Lindström, 1996), with no decrease after antipsychotic treatment. CSF dynorphin concentrations were associated with symptom severity, as well as poor clinical outcome (Heikkilä *et al.*, 1990; Lindström, 1996 but see also Zhang *et al.*, 1985). Postmortem studies have revealed no alterations in dynorphin-IR or κ receptor expression in schizophrenic patients (Hurd, 2002; Iadarola *et al.*, 1991; Peckys and Hurd, 2001).

Although a direct association between polymorphisms in the prodynorphin gene promoter and schizophrenia has not been observed, an increased risk of susceptibility to schizophrenia was associated with the Ser9Gly polymorphism in the DA D_3 receptor, particularly in individuals carrying allele 3 of the prodynorphin gene. It was suggested that prodynorphin and DA D_3 receptor genes cooperatively contribute to a background of susceptibility to the development of schizophrenia (Ventriglia *et al.*, 2002).

C. Enkephalins

δ Receptors are relatively selective for the enkephalins. The anatomical distribution of enkephalins is similar to dynorphin (Fallon and Leslie, 1986). Few studies examining the role of enkephalins in schizophrenia have been published. Decreased met-enkephalin concentrations were reported in the CSF (Burbach *et al.*, 1979; Wen *et al.*, 1983) and caudate/putamen (Kleinman *et al.*, 1985) of schizophrenic patients. Increased met-enkephalin concentrations were reported in the frontal cortex and substantia nigra, with no changes in the thalamus, or parietal and occipital cortices (Toru *et al.*, 1988).

A single mutation in the promoter region of the proenkephalin A gene was found in one schizophrenic patient, but in a larger study no more subjects with this mutation were found and its functional significance remains obscure (Mikesell *et al.*, 1997, 1996). Enkephalin-related compounds have not proven efficacious in the treatment of schizophrenia (Azorin *et al.*, 1990; de Jongh *et al.*, 1982; Jorgensen *et al.*, 1993).

VIII. Secretin

In 1902, Bayliss and Starling identified the first peptide ever discovered, secretin. Secretin, a member of the secretin/somatostatin/VIP superfamily, is composed of 27 amino acids (for review see Chey and Chang, 2003). In the brain, secretin is most abundant in the hippocampus and hypothalamus. Peripheral secretin administration increases fos protein expression in the central nucleus of the amygdala in rats (Goulet *et al.*, 2003), induces cAMP formation in the hippocampus and hypothalamus (Karelson *et al.*, 1995), and antagonizes Phencyclidine (PCP)-induced PPI disruption in rats (Myers *et al.*, 2005). In a double-blind clinical trial in which patients with refractory schizophrenia received a single intravenous injection of porcine secretin or placebo, a number of patients exhibited a transient (up to 4 days) but significant improvement in symptoms, although the overall effect was not significant (Alamy *et al.*, 2004; Sheitman *et al.*, 2004). Data from human and rodent studies suggest that the antipsychotic-like behavioral effects of secretin may be related to the effects of secretin in the limbic system (amygdala or hippocampus). Further investigation into the clinical potential of secretin in the treatment of schizophrenia is warranted.

IX. Somatostatin

Somatostatin, also known as growth hormone release-inhibiting hormone, was discovered in 1968 and five receptors have been identified, all of which are GPCRs (see Panteris and Karamanolis, 2005 for review). Two cyclic splice variants of somatostatin exist, 14 and 28 amino acids, each displaying unique tissue distributions. Somatostatin 28 is the most abundant form in the nervous system, with highest concentrations found in the olfactory tubercles, superior and inferior colliculi, and cerebellum.

The majority of CSF studies found no significant differences in CSF somatostatin concentrations in schizophrenic patients compared to controls (Banki *et al.*, 1992a,b; Gerner and Yamada, 1982; Heikkilä, 1993; Rubinow, 1986). However, reports of both increased and decreased CSF somatostatin concentrations in drug free schizophrenic patients have also been reported (Bissette *et al.*, 1986; Gerner *et al.*, 1985). Variable results have also been obtained after antipsychotic drug administration (Doran *et al.*, 1989; Sharma *et al.*, 1994). Low concentrations of CSF somatostatin were reported in schizophrenic, as well as in other psychiatric patients who were dexamethasone nonsuppressors, suggesting a functional relationship between HPA axis hyperactivity and reduced CSF somatostatin concentrations (Doran *et al.*, 1986; Rubinow, 1986). Both plasma somatostatin

concentrations (Saiz-Ruiz *et al.*, 1992) and serum somatostatin autoantibodies (Rogaeva *et al.*, 1990; Roy *et al.*, 1994) are repeatedly found to be increased in schizophrenic patients.

Postmortem studies of somatostatin in schizophrenia consistently demonstrate decreased somatostatin concentrations in the hippocampus of patients with predominant negative symptoms (Ferrier *et al.*, 1983; Roberts *et al.*, 1983). Decreased somatostatin concentrations have also been reported in cerebral cortex and lateral thalamus in schizophrenia (Gabriel *et al.*, 1996; Nemeroff *et al.*, 1983; Roberts *et al.*, 1983).

To date, no associations between somatostatin system genes and schizophrenia have been reported (Detera-Wadleigh *et al.*, 1987). Several somatostatin agonists have been approved by the FDA for the treatment of cancer, diabetes, and normalization of digestive function; however, no clinical trials in schizophrenia have been conducted.

X. Vasoactive Intestinal Peptide

The 28 amino acid peptide VIP is a potent vasodilator with a wide distribution in the CNS and in the periphery (for review see Delgado *et al.*, 2004). VIP acts on two identified receptor subtypes ($VPAC_1$ and $VPAC_2$) and historically is associated with regulation of digestive function (Harmar *et al.*, 1998). There are few studies exploring the role of VIP in schizophrenia.

No alterations in VIP concentrations have been found in the CSF of schizophrenic patients (Gjerris *et al.*, 1984; Rafaelsen and Gjerris, 1985). Postmortem studies report increased VIP concentrations in the amygdala, particularly in the central nucleus (Roberts *et al.*, 1983; Zech *et al.*, 1986). The most consistent results have been decreased VIP concentrations in the lymphocytes of schizophrenic patients, which are not altered by haloperidol treatment (Mauri *et al.*, 1998; Panerai and Sacerdote, 1993; Panza *et al.*, 1992). This observation has been postulated to be associated with the low prevalence of appendicitis in schizophrenic patients (Lauerma, 1999).

XI. Tachykinins

Mammalian tachykinins include the closely related peptides substance P (SP), neurokinin A, neurokinin B, neuropeptide K, neuropeptide γ, and hemokinin 1. The neurokinins share the common C-terminal sequence Phe-X-Gly-Leu-Met-NH_2 and are encoded by two preprotachykinin genes. All of the identified

neurokinin receptors (NK_1–NK_3) are GPCRs (see Almeida *et al.*, 2004 and Page, 2005 for review). Neurokinins and their receptors are widely and heterogeneously distributed in the CNS, particularly in cerebral cortex, NAcc, amygdala, and hypothalamus (Gale *et al.*, 1978). NK_3 is the dominant neurokinin receptor in the rat brain, whereas in the human, NK_1 is the most prevalent.

Substance P concentrations in CSF (Heikkilä *et al.*, 1990; Miller *et al.*, 1996; Rimon *et al.*, 1984) and plasma (Kaiya *et al.*, 1981) of schizophrenic patients do not differ from controls. In postmortem studies, NK_1 receptor-IR is increased in the prefrontal cortex (Tooney *et al.*, 2001). SP concentrations are increased in the prefrontal cortex, thalamus, hippocampus, and substantia nigra (Roberts *et al.*, 1983; Toru *et al.*, 1988) and decreased in the amygdala (Carletti *et al.*, 2005; Toru *et al.*, 1988). Additionally, decreased preproptachykinin A mRNA expression was reported in the amygdala (Carletti *et al.*, 2005). Two studies failed to find associations between SP, or angiotensin converting enzyme (likely the enzyme largely responsible for SP degradation in the CNS) and susceptibility to schizophrenia (Arinami *et al.*, 1996; Detera-Wadleigh *et al.*, 1987).

Because activation of NK_3 receptors increases the firing rate of DA cells in the VTA and subsequent DA release in the ventral striatum, it has been hypothesized that NK_3 receptor antagonists may possess antipsychotic-like behavioral effects (Marco *et al.*, 1998; Nalivaiko *et al.*, 1997; Seabrook *et al.*, 1995) and two clinical trials have demonstrated clinical efficacy of NK_3 receptor antagonists. The nonpeptide NK_3 receptor antagonist Talnetant (GlaxoSmithKline) was effective at improving positive and possibly cognitive symptoms in schizophrenic patients in a phase II clinical trial (Spooren *et al.*, 2005). Similarly, the nonpeptide NK_3 receptor antagonist Osanetant (SR 142801) improved total BPRS scores and positive symptoms in patients with schizophrenia and schizoaffective disorder in a multiarm clinical trial (Meltzer *et al.*, 2004). Both drugs were well tolerated and their side effects did not differ from those of placebo. Additional studies with both agents are currently underway.

XII. Thyrotropin-Releasing Hormone

The best characterized function of TRH is regulation of the hypothalamus-pituitary-thyroid (HTP) axis. TRH is associated with two GPCR subtypes; TRH-R1 and TRH-R2. TRH is found in the olfactory bulbs, piriform and entorhinal cortices, hippocampus, amygdala, NAcc, olfactory tubercle, parvocellular portion of the PVN and the periaqueductal central gray. In addition to a central role in the regulation of the HPT axis, TRH has been hypothesized to play a role in regulation of autonomic functions, arousal and cognition, locomotion, and water intake (for review see Prokai, 2002).

The TRH stimulation test assesses HPT axis function by measurement of thyroid stimulating hormone (TSH) secretion after intravenous administration of a standard dose of TRH. The TRH-induced TSH response is blunted in a significant proportion (25–33%) of depressed, alcoholic, and personality disorder patients (for review see Loosen, 1985). The frequency of a blunted TSH response to TRH in schizophrenic patients is lower than that observed in other psychiatric groups and has been associated with favorable antipsychotic drug response (Braddock and Blake, 1981; Garver, 1988; Langer *et al.*, 1986; Yazici *et al.*, 2002). No difference has been found in CSF TRH concentrations between schizophrenic patients and controls (Banki *et al.*, 1992a,b; Gjerris *et al.*, 1985; Sharma *et al.*, 2001). The only positive finding in postmortem studies of schizophrenic patients is decreased TRH-IR in the frontal cortex (Biggins *et al.*, 1983; Nemeroff *et al.*, 1983). Currently, there are no genetic studies of TRH polymorphisms in schizophrenia.

Clinical trials with TRH or its analogues have produced variable results, including exacerbation of symptoms (Bigelow *et al.*, 1975; Davis *et al.*, 1975; Wilson *et al.*, 1973), absence of therapeutic effects (Clark *et al.*, 1975; Lindström *et al.*, 1977) and improvement of positive and negative symptoms and emotionality (Brambilla *et al.*, 1986; Inanaga *et al.*, 1978; Kobayashi *et al.*, 1980; Mizuki *et al.*, 1985, 1986; Prange *et al.*, 1979).

XIII. Other Peptides

On the basis of animal data, clinical studies have investigated the role of galanin, hypocretin/orexin, arginine-vasopressin, oxytocin, and LHRH in schizophrenia. Galanin-IR is reportedly reduced in the temporal (Frederiksen *et al.*, 1991) but not frontal or occipital cortex (Sharma *et al.*, 1994) of schizophrenic patients.

The close anatomical association between hypocretin/orexin and the mesolimbic DA system was the basis for exploration of this peptide system in schizophrenia. Whereas no differences in CSF hypocretin concentrations were found between schizophrenic patients and control subjects, CSF hypocretin concentrations were significantly correlated with sleep latency in schizophrenics, one of the most consistent sleep abnormalities in schizophrenia (Nishino *et al.*, 2002). A study found decreased CSF hypocretin in patients with schizophrenia treated with haloperidol, but not atypical antipsychotic drugs, compared to unmedicated subjects (Dalal *et al.*, 2003). Additionally, a single nucleotide polymorphism in the hypocretin 1 receptor gene was associated with polydipsia-hyponatremia in schizophrenia, a not uncommon condition that appears after years of antipsychotic

drug treatment (Meerabux *et al.*, 2005). Overall, these findings suggest that hypocretin may be associated with antipsychotic drug response.

The nonapeptide arginine-vasopressin (AVP) plays a crucial role in the control of water balance in humans. Considerable attention has been given to AVP in relation to the common prevalence of hyponatremia in schizophrenic patients (3–5%) who develop potentially fatal episodes of water intoxication associated with impaired water secretion (de Leon *et al.*, 1994). Schizophrenic patients, particularly the subset that develops hyponatremia, display increased AVP plasma concentrations (Delva *et al.*, 1990; Ryan *et al.*, 2004) and alterations in AVP regulation (Goldman *et al.*, 1996; Kishimoto *et al.*, 1989; Ohsawa *et al.*, 1993). Additionally, water intoxication crises and enhanced AVP release often coincide with psychotic exacerbations (Goldman *et al.*, 1997). CSF studies have found no difference in AVP between schizophrenic patients and controls (Gjerris *et al.*, 1985; Glovinsky *et al.*, 1994; Sorensen *et al.*, 1985). Decreased AVP content was reported in the temporal cortex, but not the hypothalamus, of schizophrenic patients (Frederiksen *et al.*, 1991).

The neurohypophyseal peptide oxytocin, best known for its role in parturition, lactation, and regulation of social behavior, has also been studied in schizophrenic patients. CSF oxytocin levels were increased in drug naïve patients and increased with antipsychotic drug treatment (Beckmann *et al.*, 1985 but also see Glovinsky *et al.*, 1994). One investigator has reported improvement of psychotic symptoms in open clinical trials with oxytocin (Bujanow, 1972).

Luteinizing hormone-releasing hormone (LHRH), (gonadotropin-releasing hormone (GnRH) or gonadorelin, controls sex hormones and regulates reproductive behavior in humans and several mammals. Normal basal secretion of gonadorelin has been reported in men, women, and adolescent schizophrenic patients (Apter *et al.*, 1983; Brown *et al.*, 1995; Gil-Ad *et al.*, 1981). However, increased response of growth hormone (Gil-Ad *et al.*, 1981) and LH (Brambilla *et al.*, 1976 but also see Brown *et al.*, 1995), to LHRH challenge has also been described. The effect of antipsychotic drug treatment on the response to LHRH is unclear (Apter *et al.*, 1983; Brambilla *et al.*, 1976; Gil-Ad *et al.*, 1981; Naber *et al.*, 1980). No postmortem studies or clinical trials involving LHRH have been published.

Neuregulin 1 (NRG1) is part of epithelial growth factor family and is associated with the erB receptors. Neuregulin has crucial roles in neurodevelopmental processes, including neuronal migration, myelination, hormonal control of puberty, synaptic plasticity, and regulation of neurotransmitter expression and signaling (Corfas *et al.*, 2004; Owen *et al.*, 2005). An initial report from a genome-wide scan identified a haplotype in the 5′-end of the NRG1 gene with a highly significant association with schizophrenia in an Icelandic population (Stefansson *et al.*, 2002). Subsequently, NRG1 has been repeatedly found to be a susceptibility

gene for schizophrenia in subjects of European (Bakker *et al.*, 2004; Corvin *et al.*, 2004; Green *et al.*, 2005; Norton *et al.*, 2006; Petryshen *et al.*, 2005; Stefansson *et al.*, 2003; Williams *et al.*, 2003), Asian (Fukui *et al.*, 2006; Li *et al.*, 2004, Liu *et al.*, 2005; Tang *et al.*, 2004; Yang *et al.*, 2003; Zhao *et al.*, 2004), and African descent (Lachman *et al.*, 2006) (but also see (Hong *et al.*, 2004; Iwata *et al.*, 2004; Kampman *et al.*, 2004; Thiselton *et al.*, 2004). Additionally, the NRG1 gene has been associated with poor response to antipsychotic drugs (Kampman *et al.*, 2004) and susceptibility to bipolar disorder (Green *et al.*, 2005; Tkachev *et al.*, 2003). Furthermore, NRG1 gene is suspected to interact with erB4 to increase susceptibility to schizophrenia (Norton *et al.*, 2006). In agreement with these genetic studies are reports of decreased expression or altered expression of NRG1 (Hashimoto *et al.*, 2004) and its receptor erB3 (Hakak *et al.*, 2001; Tkachev *et al.*, 2003) and erB4 (Silberberg *et al.*, 2006) in the prefrontal cortex of schizophrenic patients. Overall, NRG1 despite its relatively recent identification, is the one with the strongest genetic data in schizophrenia and its study promises to greatly expand our understanding of this disease.

XIV. Conclusions

A clear role for neuropeptides in the etiology, pathophysiology, and treatment response of schizophrenia has not been consistently demonstrated. Factors contributing to the negative clinical data include the lack of identifiable subgroups of schizophrenic patients, the inherent heterogeneity associated with human studies (including low subject number and heterogeneity of the patient population), and inadequate methods for evaluation of peptide systems antemortem.

In postmortem studies, the frontal and temporal cortices show the most consistent abnormalities in neuropeptides and neuropeptide receptors in schizophrenic patients. These abnormalities are primarily of a quantitative (variation in the concentration of peptides or their receptors) rather than qualitative (variation in the distribution of peptides or their receptors) nature. Despite substantial preclinical evidence suggesting that neuropeptide systems in the mesolimbic pathways (specifically in the NAcc and VTA) may be involved in the pathophysiology of schizophrenia, postmortem data do not support this hypothesis.

Overall, genetic evidence of neuropeptide abnormalities in schizophrenia is weak, with the possible exception of the association between polymorphisms in the NRG1 gene and schizophrenia, as well as in the CCK_A receptor gene and positive symptoms. Ultimately, polymorphisms in the promoter regions, rather than in processing regions (i.e., exons and introns), of neuropeptide genes may prove more likely to be associated with the degree of gene expression and with the pathogenesis of schizophrenia (Tachikawa *et al.*, 2000, 2001).

Numerous agonist and antagonist ligands for neuropeptide receptors have been developed and tested for clinical efficacy in the treatment of schizophrenia. Although the majority of clinical trials are negative (possibly due to low sample size, short treatment duration, and the use of treatment-resistant populations) data support further development and testing of NK_3 receptor antagonists, and possibly secretin, TRH, and CCK_A and NT receptor agonists. In addition to larger controlled clinical trials to replicate and extend these findings, the use of psychopharmacogenetics would allow identification of specific subgroups of patients that would benefit from specific treatments. Additionally, although data support the testing of peptide agonists within several peptide systems, neuropeptide receptor antagonists seem to be the prime pharmacological targets to pursue, due to the greater availability of small molecule nonpeptide antagonists (with greater stability and ability to cross the blood-brain barrier) and reduced likelihood of developing receptor downregulation and tachyphylaxis.

Acknowledgments

The authors are supported by NIH MH-39415, MH-42088, and MH-58922. In addition, the authors' would like to disclose the following relationships. Nemeroff—Grants/Research: Abbott Laboratories, AFSP, AstraZeneca, Bristol-Myers Squibb, Eli Lilly, Forest Laboratories, GlaxoSmithKline, Janssen Pharmaceutica, Merck, NARSAD, NIMH, Pfizer Pharmaceuticals, Stanley Foundation/NAMI, Wyeth-Ayerst; Consultant: Abbott Laboratories, Acadia Pharmaceuticals, AstraZeneca, Bristol-Myers Squibb, Corcept, Cypress Biosciences, Cyberonics, Eli Lilly, Forest Laboratories, GlaxoSmithKline, Janssen Pharmaceutica, Merck, Neurocrine Biosciences, Novartis, Organon, Otsuka, Sanofi, Scirex, Somerset, Wyeth-Ayerst; Speakers Bureau: Abbott Laboratories, AstraZeneca, Bristol-Myers Squibb, Eli Lilly, Forest Laboratories, GlaxoSmithKline, Janssen Pharmaceutica, Organon, Otsuka, Pfizer Pharmaceuticals, Wyeth-Ayerst; Stockholder: Corcept, Neurocrine Biosciences; Patents: Method and devices for transdermal delivery of lithium (US 6,375,990 B1), Method to estimate serotonin and norepinephrine transporter occupancy after drug treatment using patient or animal serum (provisional filing April 2001).

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BRAIN-DERIVED NEUROTROPHIC FACTOR IN SCHIZOPHRENIA AND ITS RELATION WITH DOPAMINE

Olivier Guillin,* Caroline Demily,*,† and Florence Thibaut*,†

*Unité de Psychiatrie, UFR de Médecine et de Pharmacie de Rouen, France
†CHU Ch. Nicolle et Centre Hospitalier du Rouvray, INSERM U614, UFR de Médecine et de Pharmacie de Rouen, France

The brain-derived neurotrophic factor (BDNF) belongs to the neurotrophins family and has a role in proliferation, differentiation of neurons but also as a neurotransmitter. This neurotrophin has received much attention during the last year in regard of the pathophysiology of schizophrenia. Results of genetic studies conducted in schizophrenia support a role for BDNF in schizophrenia and in brain function associated with the disorder. The changes of BDNF observed in the brain and in the plasma of patients with schizophrenia have generated results that can be interpreted either as a hallmark of the disease or a consequence of antipsychotic drugs. Antipsychotic drugs act by blocking the dopamine transmission at the dopamine D2-like receptors. BDNF controls the expression of one of these D2-like receptors, the dopamine D3 receptor. This raises the hypothesis of a link between cortical area, via BDNF, and the dopamine neurotransmission pathway in schizophrenia and its treatment.

I. Introduction

The brain-derived neurotrophic factor (BDNF) belongs to the neurotrophins family, which comprises the prototypical member nerve growth factor (NGF), neurotrophine-3 (NT-3), and neurotrophine-4/5 (NT-4/5). NGF was initially

INTERNATIONAL REVIEW OF NEUROBIOLOGY, VOL. 78
DOI: 10.1016/S0074-7742(06)78012-6

0074-7742/07 $35.00

identified as being responsible for proliferation, differentiation, and function of sympathetic nerve cells (Levi-Montalcini and Cohen, 1960). Neurotrophins can act after their neuronal uptake and retrograde transport to the soma, through their high-affinity tropomyosine-related tyrosine (Trk) receptors and the low-affinity $p75^{NTR}$ receptor, a member of the tumor necrosis factor (TNF) receptor family (Thoenen, 1995). Trks display specificity for the neurotrophins: NGF only binds with high affinity to TrkA, NT-3 to TrkC and TrkB, BDNF, and NT-4/5 to TrkB. These receptors possess an intracellular tyrosine kinase domain, which transduce the neurotrophins signal by autophosphorylation and subsequent recruitment of enzymes such as phosphatidylinositol-3 kinase or adaptor proteins such as ShC linked to various serine/threonin kinases (Thoenen, 1995).

It was admitted that, whereas interaction with the Trk receptor promotes cell survival, interaction with $p75^{NTR}$ promotes cell death. Data have challenged this overly simplistic dual scheme (Kalb, 2005; Lu *et al.*, 2005). Neurotrophins are synthesized in a precursor form (proneutrophins), which generate the mature neurotrophins through proteolytic cleavage. Proneurotrophins bind with higher affinity to $p75^{NTR}$ than do mature neurotrophins, but they do not bind to Trk receptors (Lee *et al.*, 2001). Moreover, complexes of $p75^{NTR}$, or homologues of this receptor (Kanning *et al.*, 2003) with either sortilin or Trk receptor modulate the affinity of the neurotrophins (Kalb, 2005). It results form these considerations that life–death decisions in neurons depend not only on neurotrophic supply but also on the pro- and mature-neurotrophin balance (Lu *et al.*, 2005).

A more diverse role for BDNF as an extracellular transmitter has, nevertheless, been inferred from observations that it is anterogradely transported (Altar *et al.*, 1997; von Bartheld *et al.*, 1996), released on neuron depolarization, and triggers rapid intracellular signals (Altar and DiStefano, 1998; Thoenen, 1995) and action potentials in central neurons (Kafitz *et al.*, 1999) via intracellular transduction of its high-affinity membrane receptor TrkB (Blum *et al.*, 2001). BDNF can alter fast synaptic transmission by speeding up the development of excitatory and inhibitory synapses (Vicario-Abejon *et al.*, 1998) but also by modulating synaptic efficacy (Huang *et al.*, 1999; Lohof *et al.*, 1993). In particular, BDNF is necessary for the induction and maintenance of hippocampal long-term potentiation (Barco *et al.*, 2005; Figurov *et al.*, 1996; Korte *et al.*, 1995; Kovalchuk *et al.*, 2002; Patterson *et al.*, 1996). ProBDNF demonstrate opposite function in activating p75(NTR) which facilitates hippocampal long-term depression (Woo *et al.*, 2005). As suggested in life–death decision, a bidirectional regulation of synaptic plasticity by proBDNF and mature BDNF might exist. Although some observations suggest a role of BDNF in nociception (Kerr *et al.*, 1999), mechanosensation (Carroll *et al.*, 1998), and learning (Du and Poo, 2004; Egan *et al.*, 2003; Lee *et al.*, 2004; Linnarsson *et al.*, 1997; Minichiello *et al.*, 1999).

For more than 40 years, dopamine has been consistently implicated in the pathophysiology of schizophrenia and its treatment (Carlsson, 1995). Evidences

have emerged showing BDNF-induced synaptic plasticity and modulating the physiological functions of the dopamine transmission pathway (Berton *et al.*, 2006; Goggi *et al.*, 2003; Grimm *et al.*, 2003; Guillin *et al.*, 2001; Horger *et al.*, 1999; Monteggia *et al.*, 2004).

In this chapter, we propose to review evidences generated by genetic, plasma, and brain concentration of BDNF studies in the human affected by schizophrenia and the relationship between dopamine and BDNF.

II. Genetic Studies

The BDNF gene is localized on the reverse strand of chromosome 11p13 and encodes a precursor peptide (proBDNF), with a strong conservation of the coding sequence across species. The gene consists of four short 5′ exons with separate promoters and one 3′ exon encoding mature BDNF protein (Metsis *et al.*, 1993; Timmusk *et al.*, 1993).

In schizophrenia, no significant linkage has been reported in this region in the large genome-wide scan studies. In bipolar disorders, a linkage study has generated an LOD score of 1.89 in bipolar families (Detera-Wadleigh *et al.*, 1999). Genetic association studies have been carried out in order to determine and define the association between polymorphisms located in the BDNF gene and schizophrenia.

In 1992, the first study by Pröschel *et al.* (1992) described a microsatellite: GT dinucleotide repeat polymorphism (166–174 bp) in the human BDNF gene, upstream from the transcription start site of the 1.6-kb BDNF mRNA transcript (Pröschel *et al.*, 1992). Many studies have been conducted in order to find a possible association between this polymorphism and schizophrenia (Hawi *et al.*, 1998; Krebs *et al.*, 2000; Sasaki *et al.*, 1997; Virgos *et al.*, 2001; Wassink *et al.*, 1999). No association was found between this polymorphism and schizophrenia, except for the Italian family study (Muglia *et al.*, 2003). A French study found no statistical difference in allele or genotype distribution of this polymorphism between schizophrenic patients and controls (Krebs *et al.*, 2000). However and interestingly, the authors revealed an excess of the 172- to 176-bp alleles in late onset, neuroleptic-responding patients ($n = 68$ patients) and in nonsubstance abuse patients ($n = 52$ patients).

BDNF plays an important role in activity-dependent hippocampal neuroplasticity and hippocampal-dependent memory. The valine-to-methionine variation at codon 66 of BDNF coding sequence, located in the 5′ proregion of the BDNF protein, is a frequent single nucleotide missense and functional polymorphism in the human BDNF gene (Egan *et al.*, 2003). The presence of the

Val66Met has been associated with abnormal intracellular trafficking and activity-dependent secretion of BDNF in cultured hippocampal neurons (Egan *et al.*, 2003). In fact, both lower depolarization-induced secretion and failure to localize to secretory granules or synapses were demonstrated for 66Met BDNF-transfected neurons using virus-mediated transfection in cultured hippocampal neurons, compared with 66Val BNDF (Egan *et al.*, 2003).

In human subjects, the Met allele is associated with impaired episodic memory assessed with the Wechsler Memory Scale (WMS) (Egan *et al.*, 2003; Tan *et al.*, 2005a). However, these data have only been partially replicated, since schizophrenic patients and relatives were analyzed separately: 66Met was associated with a lower score at the WMS but only in relatives (Dempster *et al.*, 2005). The Met carriers (healthy subjects) exhibit relatively diminished hippocampal activity in comparison with the Val/Val subjects during both encoding and retrieval processes. This impairment was directly linked to abnormal hippocampal activation: Val/Met individuals have an abnormal pattern of increased BOLD fMRI signal activation of bilateral caudal hippocampus. In contrast, Val/Val subjects show a characteristic hippocampal deactivation pattern (Egan *et al.*, 2003). A direct effect of BDNF alleles on hippocampal processing of memory was then demonstrated when the same group showed that the interaction between the BDNF Val66Met genotype and the hippocampal response during encoding accounted for 25% of the total variation in recognition memory performance (Hariri *et al.*, 2003). Moreover, this polymorphism in the BDNF gene affects the morphology of the brain: Met-carriers have a reduction in hippocampal gray matter and in the dorsolateral prefrontal cortex compared to the Val-carrier (Pezawas *et al.*, 2004). Significant interaction between prefrontal cognitive performance (the N-back test) and Val/Val genotype implicates that working memory could also be affected by the BDNF Val66Met polymorphism (Rybakowski *et al.*, 2006). Moreover, Val66Met seems to be associated with age-related change in reasoning skills. In a cohort of healthy subjects, aged 79 years at the time of the study whom reasoning was assessed by the Raven's progressive, Met homozygotes scored significantly higher than heterozygotes and Val homozygotes (Harris *et al.*, 2006).

A study has examined a large schizophrenia sample ($n = 321$) in comparison with bipolar patients ($n = 321$) and controls ($n = 350$), testing haplotype frequencies for the BDNF GT dinucleotide-repeat and the Val66Met polymorphisms (Neves-Pereira *et al.*, 2005). The authors underlie a significant excess of the Val haplotype in schizophrenics without stratification bias and suggest that Met or Met combined with the 174-bp haplotypes may be a protective factor against schizophrenia. In a large sample of 94 families, a transmission disequilibrium test (TDT) showed a preferential transmission of the Val allele from the heterozygous parents (Val/Met) to their affected schizophrenic offspring (Rosa *et al.*, 2006).

However, no association was found between the prefrontal tests assessed (WMS) and the BDNF Val/Met polymorphism in this sample (Rosa *et al.*, 2006).

Three single nucleotide polymorphisms (rs3750934, rs6265, and ro. 000001) have been investigated in an association study in the Chinese population (Chen *et al.*, 2006). No significant differences were found for either the genotype or allele distribution of analyzed polymorphisms.

BDNF and dopamine D3 receptor (DRD3) interplay might be of special interest in schizophrenia (Guillin *et al.*, 2004). Interestingly, an interaction between BDNF Met-containing haplotypes and DRD3 receptor ser/ser haplotypes has been found in early age at onset of schizophrenia (Gourion *et al.*, 2005).

Association between BDNF polymorphisms and response to antipsychotic treatment or tardive dyskinesia have been also investigated. An association between the BDNF gene Val66Met polymorphism and a good response to clozapine treatment in schizophrenia was found (Hong *et al.*, 2003). Association and gene–gene interaction between the DRD3 ser^{9}gly and BDNF Val66Met polymorphisms have been investigated in a cohort of patients with schizophrenia who had significantly high abnormal involuntary movements (Liou *et al.*, 2004). Heterozygoty for the BDNF genotype was associated with abnormal orofacial movement scores but neither DRD3 nor BDNF genotypes were clearly associated with tardive dyskinesia and no gene interaction was found.

A single nucleotide substitution (C270T) in the 5′ noncoding BDNF region was described and a significant association with late-onset Alzheimer disease and T270 was found (Kunugi *et al.*, 2001). Comparing schizophrenic patients (n = 178) and controls (n = 332), the frequency of this nucleotide substitution was significantly increased in schizophrenia (Nanko *et al.*, 2003). The association was replicated in another sample. The C/T genotype was overrepresented in schizophrenics (n = 101, 25.7%) compared to controls (n = 68, 5.9%), despite a heterogeneity between populations (Szekeres *et al.*, 2003). However, another study has found no association between schizophrenia and the C270T polymorphism in the BDNF gene for genotype and allelic distribution (Szczepankiewic *et al.*, 2005). Galderisi *et al.* (2005) have studied two polymorphisms: COMT Val^{158}Met and BDNF C270T in patients with schizophrenia versus controls. This case control association study does not report evidence for association between these polymorphisms and schizophrenia. The functional significance of C270T substitution in the promoter region of BDNF is not clear and there is no evidence that the C270T is involved in alterations of protein expression or function.

In conclusion, BDNF gene is a relatively new and promising target in genetic studies of mental disorders. Concerning schizophrenia, the initial results need replication. However, considering a polygenic model of schizophrenia transmission, BDNF can be involved in a genetic modulation pathway.

III. BDNF in the Serum of Patients with Schizophrenia

BDNF can be detected in the plasma of patients with schizophrenia and controls. To date, seven studies (summarized in Table I) have investigated BDNF plasma levels in patients with schizophrenia, drug free (Pirildar *et al.*, 2004; Shimizu *et al.*, 2003), drug naïve, with substance abuse (Jockers-Scherubl *et al.*, 2004; Pirildar *et al.*, 2004), chronically treated with antipsychotics drugs (Jockers-Scherubl *et al.*, 2004; Pirildar *et al.*, 2004; Shimizu *et al.*, 2003; Tan *et al.*, 2005b,c; Toyooka *et al.*, 2002) and the relationship with the presence of tardive dyskinesia (Tan *et al.*, 2005c). In drug-naïve patients no change in BDNF plasma levels was found (Jockers-Scherubl *et al.*, 2004; Shimizu *et al.*, 2003) whereas conflicting results have emerged from studies with drug-free patients: in one study BDNF plasma levels was decreased (Pirildar *et al.*, 2004) and there was no change in the second one (Huang and Lee, 2005). In patients with schizophrenia treated with antipsychotic drugs at the time BDNF plasma levels were determined, decreased (Grillo *et al.*, 2007; Tan *et al.*, 2005b,c; Toyooka *et al.*, 2002), or normal (Shimizu *et al.*, 2003) plasma levels compared to controls were observed. Interestingly, tardive dyskinesia (Tan *et al.*, 2005c) and substance abuses (Jockers-Scherubl *et al.*, 2004) have been found to be associated with decreased BDNF plasma levels in patients with schizophrenia.

Altogether these results seem to indicate that BDNF plasma levels are decreased in medicated patients with schizophrenia. However, the source of BDNF found in the plasma is still unknown as the relationship between plasma and brain levels. Nevertheless, brain- and plasma-level changes are correlated during aging in rats (Karege *et al.*, 2002). One way to explain the conflicting results between plasma levels found in patients chronically treated with antipsychotic drugs and drug-naïve patients might be the possible ability of antipsychotic drugs to decrease BDNF expression (Section IV).

IV. BDNF and TrkB Receptor in the Brain of Patients with Schizophrenia

Several studies have been conducted in order to determine BDNF protein or mRNA and TrkB mRNA levels in the postmortem brain tissue from patients with schizophrenia.

In the first study to be published, a significant increase of BDNF protein, determined by immunoassay, was found in the anterior cingulate and the hippocampus (Takahashi *et al.*, 2000). This was confirmed in another collection of brain tissue in the cortical areas but not in the hippocampus (Durany *et al.*, 2001).

TABLE I
BDNF Plasma Levels in Schizophrenia

Author	*N* controls	*N* patients	Treatment Status	Controls (*n.* mean ± SD)[a]	Patients (*n.* mean ± SD)[a]	Patients subtype (*n.* mean ± SD)[a]	*p*	Effect size[b]
Tan *et al.* (2005b)	45	81	M	100 ± 43	74 ± 26		<0.001	0.6
Tan *et al.* (2005c)	45	125	M	100 ± 43	66 ± 26[3]	55 ± 17[4]	[3]vs controls = 0.002 [4]vs controls <0.01	[3]0.8 [4]1.05
Jockers-Scherubl *et al.* (2004)	72	157	DN	100 ± 40	99 ± 45	134 ± 55[5] 129 ± 45[6]	[5]vs controls = 0.0062 [6]vs controls = 0.0131	[5]0.85 [6]0.725
Pirildar *et al.* (2004)	22	22	DF[1]	100 ± 35	53 ± 30		<0.001	1.34
Shimizu *et al.* (2003)	40	40	15 DN 25 M	100 ± 32	84 ± 28[7]	98 ± 43[8]	NS	0.5
Toyooka *et al.* (2002)	62	2 independent groups of 34	M	100 ± 67	55 ± 30		0.004	0.67
Huang and Lee (2005)	96	126	DF[2]	100 ± 48	100 ± 49		NS	

[a]Mean normalized to mean of control subjects.

[b]Effect size calculated as (mean patients − mean controls)/SD controls.

M, patients taking antipsychotics drugs at the time of the study; DN, drug naïve; DF, drug free for at least 2 weeks[1] or 1 week[2].

Patients with schizophrenia without[3] and with tardive dyskinesia[4]; with cannabis intake[5] or cannabis and additional substances[6]; drug naïve,[7] or medicated[8].

NS, nonsignificant.

A very comprehensive paper using three different methods to determine BDNF levels, RNAse protection assay, Western blotting, and *in situ* hybridization was published 3 years later (Weickert *et al.*, 2003). In this study, BDNF mRNA and protein was decreased in the lateral prefrontal cortex of patients with schizophrenia, especially in the layers III, V, and VI. This result was confirmed by another group in two independent cohorts (Hashimoto *et al.*, 2005). Combining the two independent cohorts, the authors found in the prefrontal cortex a 35% decrease of BDNF mRNA in layers II, III, and V/VI. In the same sample of postmortem brain tissue, TrkB mRNA was found to be decreased in the prefrontal cortex by 23% (Hashimoto *et al.*, 2005). The same result was found in the brain collection of the NIMH in the dorsolateral prefrontal cortex (Weickert *et al.*, 2005).

To date, two from four studies indicate that in patients with schizophrenia BDNF and TrkB are downregulated. Could decreased BDNF in some of the studies be attributable to antipsychotic drugs? First, in one of the studies showing a decrease of BDNF, any correlation between lifetime antipsychotic exposures and BDNF levels was found (Weickert *et al.*, 2003). Moreover, in the other one, some patients were free of medication for more than 1 month at the time of death and did not demonstrate significant higher expression of BDNF in the prefrontal cortex than patient under treatment (Hashimoto *et al.*, 2005). However and interestingly, in the study by Takahashi *et al.* (2000) showing an increase of BDNF in the cingulate cortex, patients were treated at the time of death with very low doses of antipsychotics as reflected by an average equivalent chlorpromazine of the sample of 72 mg/day.

Antipsychotic drugs' effects on BDNF expression in the brain of normal rats and nonhuman primates have been investigated. Haloperidol, a first-generation antipsychotic drug, given for more than 9 months in monkeys do not induce significant changes in BDNF mRNA in the prefrontal cortex (Hashimoto *et al.*, 2005). Other studies, conducted in normal rats have found no change or decreased BDNF mRNA and protein in cortical regions after chronic exposure to antipsychotic drugs (Angelucci *et al.*, 2000; Dawson *et al.*, 2001; Linden *et al.*, 2000; Lipska *et al.*, 2001; Nibuya *et al.*, 1995; Takahashi *et al.*, 2000).

Dysfunction of glutamatergic neurotransmission has been proposed to play an important role in the pathophysiology of schizophrenia (Goff and Coyle, 2001). In rats, glutamate hypofunction induced by the administration of MK-801 induces an increase of BDNF mRNA in the cortex which is circumvented by clozapine or haloperidol pretreatment (Linden *et al.*, 2000; Sokoloff *et al.*, 2006).

At this time, the more conservative conclusion of postmortem studies on BDNF expression in the prefrontal cortex is that there is discrepancy between studies and that the role of treatment on the results is still under debate.

V. Dopamine–BDNF Interactions

Since the 1960s, a crucial role for dopamine in schizophrenia and its treatment was suspected in schizophrenia (Carlsson and Lindqvist, 1963). It has been confirmed by imaging studies (Abi-Dargham and Laruelle, 2005; Laruelle, 2000, 2005). Some recent evidences link BDNF and dopamine neurotransmission.

A. BDNF Supports the Survival and the Differentiation of Dopaminergic Neurons

BDNF is expressed in the adult dopaminergic neurons of the midbrain and destruction of dopaminergic cells by 6-hydroxydopamine result in a loss of BDNF mRNA (Seroogy *et al.*, 1994). In the same line of evidences, in disease with a loss of dopaminergic neurons like Huntington disease and Parkinson disease postmortem studies have shown a decrease of BDNF in the substancia nigra and the striatum (Chauhan *et al.*, 2001; Ferrer *et al.*, 2000; Howells *et al.*, 2000; Mogi *et al.*, 1999; Parain *et al.*, 1999). Using neurons in culture, BDNF was found to promote the survival of the dopaminergic neurons of the developing substancia nigra (Hyman *et al.*, 1991) and to elicit an increase in the depolarization-induced release of dopamine (Feng *et al.*, 1999). In mice lacking selectively the BDNF gene in the midbrain, there is a significant but not complete reduction in non-expressing calbindin and calcineurin dopaminergic neurons during development (Baquet *et al.*, 2005). However, this has not been confirmed in a report (Berton *et al.*, 2006). Moreover, BDNF protects dopaminergic neurons from the toxic effect of MPTP, 6-hydroxydopamine, oxidative stress, and hypoglycemic injury (Hung and Lee, 1996; Levivier *et al.*, 1995; Nakao *et al.*, 1995; Petersen *et al.*, 2001; Shults *et al.*, 1995). These functions are likely to complement and overlap with those of other neurotrophic factor, including glial cell line-derived neurotrophic factor and bone morphogenetic proteins, which are also known to enhance differentiation and survival of dopaminergic neurons (Brederleau *et al.*, 2002; Feng *et al.*, 1999; Gratacos *et al.*, 2001; Zuch *et al.*, 2004).

B. BDNF in the GABA-Containing Local Circuit Neurons of the Prefrontal Cortex

Alteration in the circuitry of the dorsal prefrontal cortex appears to contribute to the working memory impairments in schizophrenia (Lewis and Lieberman, 2000), more particularly in the GABA-containing local circuit neurons of the

prefrontal cortex (Volk and Lewis, 2002), which BDNF regulates the maturation in the developing cortex (Huang *et al.*, 1999). Several lines of evidences have emerged from the Lewis's group in Pittsburgh involving BDNF in the alteration of GABA neurons in schizophrenia. These authors have shown in the postmortem brain tissue of patients with schizophrenia that the decrease in BDNF and TrkB mRNA was correlated with the decrease in GAD_{67} mRNA, a marker of the integrity of GABA neurons (Hashimoto *et al.*, 2005). However, the presence of the BDNF Met66 allele, a polymorphism which reduces the trafficking and secretion of BDNF protein (Egan *et al.*, 2003) did not contribute to the decreased level of GAD_{67} mRNA expression of the same sample of brain tissue of patients with schizophrenia (Hashimoto and Lewis, 2006).

C. Functional Interplay Between BDNF and Dopamine

The first line of evidences indicating a role of BDNF in modulating dopaminergic functions came from studies in rats infused with recombinant BDNF in the substancia nigra. In these animals, an increase of locomotor activity and dopamine agonists-induced rotational behavior is found suggesting that BDNF increases dopamine function. This was also suggested by the fact that supranigral infusion of BDNF elicits dopamine turnover in the striatum and increases the electrical activity of dopaminergic neurons via an activation of the PI3K and Ras-MEK pathways (Altar *et al.*, 1992; Goggi *et al.*, 2003; Shen *et al.*, 1994).

Chronic exposure to drug of abuse elicits long-lasting changes in the ventral tegmental area, a brain region involved in drug addiction (Koob, 1992). Rats chronically treated with morphine have a reduction in their ventral tegmental area of dopaminergic neurons that BDNF infusion prevents (Sklair-Tavron *et al.*, 1996). Chronic infusions of BDNF into the ventral tegmental area in the nucleus accumbens result in increased locomotor activity and enhanced locomotor sensitization to cocaine (Horger *et al.*, 1999).

In mice experiencing repeated aggression, a local deletion of the BDNF gene in the dopaminergic neurons of the ventral tegmental area does not permit the development of social avoidance like it does in animals with a normal expression of BDNF (Berton *et al.*, 2006b).

Altogether, these results are consistent with the idea of BDNF as a modulator of dopaminergic function.

D. BDNF Controls the Expression of the Dopamine D3 Receptor

The DRD3 belongs to the dopamine D2-like receptors (Sokoloff *et al.*, 1990). From the beginning, attention has been attracted to the restricted distribution of the DRD3 in the brain, seemingly related to functions of dopamine associated

with the limbic brain (Bouthenet *et al.*, 1991). Hence, the hypothesis has been put forward that the DRD3 receptor could be involved in the pathophysiology of schizophrenia. Several other findings might implicate the DRD3 in the pathophysiology of schizophrenia. For instance, antipsychotic drugs display affinity at recombinant dopamine D2 receptor and DRD3 in the same magnitude (Sokoloff *et al.*, 1990). Moreover, in spite of controversy (Sabate *et al.*, 1994), the meta-analysis of the ser–gly polymorphism of the DRD3 have been found to be associated with schizophrenia (Dubertret *et al.*, 1998). However, the most direct evidence of a role of the DRD3 in schizophrenia has come from a postmortem study which, at this date, has never been refuted, neither replicated. DRD3 levels have been found elevated in the brain of drug-free schizophrenic patients, but not in patients under medication with antipsychotics at the time of death (Gurevich *et al.*, 1997). This suggests that increased DRD3 expression is a hallmark of the disease and that antipsychotic medications normalize this receptor expression. DRD3 overexpression in the etiology of schizophrenia raises the question of mechanisms governing this receptor expression during development.

In adults, the expression of the DRD3 in medium-sized neurons of the nucleus accumbens, but not in granule cells of the islands of Calleja, is highly dependent on the dopaminergic innervation: ablation of the afferent neurons by unilateral 6-hydroxydopamine results in a dramatic decrease in DRD3 density in the ipsilateral nucleus accumbens (Levesque *et al.*, 1995). This paradoxical change (the dopamine D2 receptor is upregulated under these circumstances) was shown to depend on the lack of an anterogradely transported factor from dopaminergic neurons, distinct from dopamine itself and its known peptide cotransmitters, and which is released on dopamine neuron activation (Levesque *et al.*, 1995). Among the candidate factors for regulating DRD3 expression, BDNF was particularly attractive, since it is expressed by dopamine neurons (Seroogy *et al.*, 1994). BDNF immunoreactivity is prominent in the shell of the nucleus accumbens of normal rats (Conner *et al.*, 1997), and its receptor, the TrkB, colocalizes with the DRD3 (Guillin *et al.*, 2001).

Several lines of evidences have lead to the conclusion that BDNF controls DRD3 expression (Guillin *et al.*, 2001). In mice with a BDNF-null mutation, DRD3 binding and mRNA are low at postnatal days 9–14 and do not increase at later stages as it does in their normal littermates. These results show that BDNF is required for the normal development of DRD3 expression in the shell of the nucleus accumbens. In unilaterally 6-hydroxydopamine-lesioned rats, repeated administration of levodopa, leading to extraneuronal dopamine formation, triggers DRD3 overexpression, not only in the shell of the nucleus accumbens but also in the denervated striatum, a brain structure in which DRD3 expression is hardly detectable (Bordet *et al.*, 1997). During levodopa treatment of 6-hydroxydopamine-lesioned rats, infusion into the denervated striatum of a selective BDNF antagonist impairs induction of both DRD3 mRNA and protein

expression (Guillin *et al.*, 2001). This overexpression of the DRD3 in the denervated striatum triggers the development of behavioral sensitization to levodopa (Bordet *et al.*, 1997). Infusion of the selective TrkB antagonist dose dependently inhibits behavioral sensitization, indicating that behavioral sensitization is triggered by BDNF (Guillin *et al.*, 2001). Striatal BDNF in fact originates mainly from cortical neurons (Altar *et al.*, 1997). In agreement, cortical ablation partially impairs the induction of DRD3 overexpression in the striatum and behavioral sensitization, indicating that both processes require the participation of corticostriatal neurons (Guillin *et al.*, 2001). Levodopa also induces BDNF mRNA on the frontal cortex in the 6-hydroydopamine-lesioned side, mainly in cortical layer V, containing pyramidal cell bodies, and in layer VI, which sends projections to various subcortical areas, notably striatal and accumbal areas. This effect critically depends on activation of the dopamine D1/D5 receptors (Guillin *et al.*, 2001).

Altogether, these results demonstrate that BDNF triggers behavioral sensitization by controlling DRD3 expression and, more generally controls dopamine tone in the limbic forebrain.

VI. Conclusions

Behavioral sensitization of the dopamine system might be involved in the early stage of schizophrenia and more likely in the pathophysiology of positive symptoms (Laruelle, 2005; Lewis and Lieberman, 2000). In schizophrenia, DRD3 protein is elevated in nontreated patients. Therefore, BDNF should be found elevated in cortical regions of nontreated patients with schizophrenia. As discussed before, these data are not available at this time as all postmortem studies were performed on brain of patients treated by antipsychotic at the time of death. Moreover, the effect of antipsychotic drugs on BDNF expression is still controversial. Thus, the view that hypo- or hyperfunction of BDNF in the prefrontal cortex participate to the emergence of symptoms of schizophrenia could be supported (Hashimoto *et al.*, 2005; Sokoloff *et al.*, 2006; Weickert *et al.*, 2003).

However, results show that subchronic blockade of glutamatergic transmission induces an increase of BDNF in the frontal cortex and a striatal DRD3 overexpression in mice, that is corrected by antipsychotic drugs (Sokoloff *et al.*, 2006). In sensitized animals, DRD3 expression is under the control of prefrontal cortex BDNF and this expression is under the control of the dopamine D1 receptor stimulation (Guillin *et al.*, 2001). Dopamine D1 expression have been found to be elevated in the dorsolateral prefrontal cortex of patients with schizophrenia and associated to working performance impairment (Abi-Dargham *et al.*, 2002).

Thus, we can hypothesized that, by an unknown neurodevelopmental process, dopamine D1 function is enhanced in the dorsolateral prefrontal cortex, leading to an increase of BDNF expression, that, in turns, elicits an overexpression of the DRD3 in the striatum that participates to the expression of positive symptoms. Antipsychotic drugs might decrease BDNF levels and, therefore, normalize subcortical dopaminergic hyperfunction mediated by the DRD3.

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SCHIZOPHRENIA SUSCEPTIBILITY GENES: IN SEARCH OF A MOLECULAR LOGIC AND NOVEL DRUG TARGETS FOR A DEVASTATING DISORDER

Joseph A. Gogos

Department of Physiology and Cellular Biophysics, and Center for Neurobiology and Behavior
Columbia University, College of Physicians and Surgeons, New York, New York 10032, USA

Schizophrenia is a devastating psychiatric disorder that affects approximately one percent of the population worldwide. We argue that the efforts to decipher the genetic causes of schizophrenia have reached another turning point and describe evidence supporting some of the major recent genetic findings in the field. In addition, we identify some general areas of caution in the interpretation of these findings and addresses the promise this recently acquired knowledge holds for the generation of reliable animal models, characterization of genetic

INTERNATIONAL REVIEW OF NEUROBIOLOGY, VOL. 78
DOI: 10.1016/S0074-7742(06)78013-8

0074-7742/07 $35.00

interactions, dissection of the disease pathophysiology and development of novel, mechanism-based treatments for the patients.

I. The Genetic Component of Schizophrenia

Schizophrenia is a severe psychiatric disorder with a lifetime prevalence of ~1% in most studied populations (Karayiorgou and Gogos, 1997). Schizophrenia is characterized by so called "positive symptoms" including delusions and hallucinations, "negative symptoms" including blunted emotions and social isolation, as well as by cognitive deficits. Although cognitive impairment has always been regarded as a hallmark feature of schizophrenia, only recently it has been recognized as an enduring, core deficit, a strong indicator of specific genetic liability to the disease and a primary target for pharmacotherapy. Individuals with schizophrenia show varying degrees of deficiency in a diverse range of cognitive domains such as working memory, short-term and episodic memory, attention, executive functions, and learning (Bowie and Harvey, 2005; Goldman-Rakic, 1994; Green *et al.*, 2004).

Similar to many common, complex disorders, schizophrenia is a multifactorial disorder characterized by the contribution of multiple risk genes, which could act in conjunction with epigenetic and environmental processes (Karayiorgou and Gogos, 1997). More than 20 genome-wide scans aiming to localize genes for this disorder have been reported to date and two meta-analyses (Badner and Gershon, 2002; Lewis *et al.*, 2003) implicated under moderate stringency, ~12 regions of the genome as likely to contain schizophrenia susceptibility genes (2p, 5q, 3p, 11q, 2q, 1q, 22q, 8p, 6p, 20p, 13q, and 14q). This is most likely to be an underestimate because it is expected that many schizophrenia susceptibility genes will be undetectable by traditional linkage studies. The ultimate validation of the linkage results is gene identification, which in turn represents an important milestone for understanding the disease pathophysiology. Gene identification has proven to be an extraordinarily difficult task, partly because no single gene is necessary or sufficient to cause the disease but instead, many susceptibility genes with small effects act in combinations to increase the risk of illness. Phenotypic heterogeneity has also contributed to the difficulties associated with genetic research in schizophrenia. Phenotypic heterogeneity is to be expected due to the complexity of the brain, but the majority of genetic studies by relying on a categorical binary diagnosis ("affected" vs "unaffected") do not take into account the possible differences in representation among different samples of the various components of the illness.

Nevertheless, in the past 4 years, significant advances in gene discovery have taken place driven by the completion of the sequencing of the human genome, the increasingly available technology for high-throughput genomic analysis, and the development of new analytical and bioinformatics tools. Several new susceptibility

genes have been proposed, each supported by varying degrees of evidence. Candidate genes have been identified, for the most part, through systematic follow-up of linkage signals involving genotyping of relatively large numbers of markers, including single nucleotide polymorphisms (SNPs) and linkage disequilibrium (LD) assays or through multipronged candidate gene approaches involving analysis of expression patterns and biological functions. Far from being a mere academic exercise, this newly acquired knowledge base provides a novel framework for mechanism-based drug discovery efforts. In some cases, susceptibility genes could themselves provide new drug targets. Alternatively, the identification of these genes will lead to improved understanding of the basis of schizophrenia pathogenesis and eventual detailed characterization of the affected molecular pathways.

Here, we discuss the available genetic and biological data behind these strong candidate genes, the statistical support for these findings, and future directions of the genetic research. In this context, we discuss the development of animal models, the characterization of susceptibility gene interactions, the understanding of the disease pathophysiology, and the development of mechanism-based therapies.

II. Genes Identified Through Systematic Follow-Up of Linkage Signals

In this section, we discuss the genetic data for recently identified strong positional candidate genes (in chronological order of appearance of the reports), as well as their possible biological functions. The first report of a strong positional candidate schizophrenia gene identified by a systematic fine-mapping approach within a region implicated by linkage analysis was published in 2002 (Liu *et al.*, 2002a) followed in the same year by three additional reports describing new susceptibility genes identified through similar approaches (Chumakov *et al.*, 2002; Stefansson *et al.*, 2002; Straub *et al.*, 2002). Additional genes have been reported based on systematic follow-up analysis of linkage peaks (Brzustowicz *et al.*, 2004; Duan *et al.*, 2004; Hennah *et al.*, 2003; Mukai *et al.*, 2004; Petryshen *et al.*, 2005a; Pimm *et al.*, 2005; Table I).

A. Proline Dehydrogenase

The gene is located on chromosome 22q11, a region implicated by some linkage studies (Badner and Gershon, 2002; Lewis *et al.*, 2003) and also frequently deleted in patients with schizophrenia (Karayiorgou *et al.*, 1995). Several studies have now established conclusively that the risk of schizophrenia for a patient with a 22q11 microdeletion is ~25–31 times the general population risk of 1% (Murphy *et al.*, 1999; Pulver *et al.*, 1994) and that the rate of 22q11 microdeletions

TABLE I
SCHIZOPHRENIA CANDIDATE GENES: CHROMOSOMAL LOCATIONS AND POTENTIAL FUNCTION

Gene symbol	Locus	Function	References
PRODH	22q11	L-Proline metabolism; influence on glutamatergic transmission, mitochondrial function	Liu *et al.* (2002a)
DTNBP1	6p22	Member of DPC and biogenesis of lysosomal related organelle complex; potential presynaptic effects on glutamate release	Straub *et al.* (2002)
NRG1	8p12	Broad involvement in neuronal function and survival	Stefansson *et al.* (2002)
G72	13q34	Potential activation of D-amino acid oxidase and indirect effects on glutamatergic signaling	Chumakov *et al.* (2002)
DISC1	1q42	Multifunctional; possible involvement in cell migration and phosphodiesterase signaling	Millar *et al.* (2000), Hennah *et al.* (2003)
CAPON	1q22	Potential regulator of NMDA receptor-coupled nitric oxide signaling	Brzustowicz *et al.* (2004)
ZDHHC8	22q11	Palmitoylation of PSD-95 and other substrates; implications for synaptic assembly and function	Liu *et al.* (2002b), Mukai *et al.* (2004)
TAAR6	6q23	G-protein–coupled receptor for trace amines	Duan *et al.* (2004)
EPN4	5q33	Potential role in reuptake and storage of neurotransmitters	Pimm *et al.* (2005)
$GABA_{(A)}$ receptors	5q34	GABAergic transmission	Petryshen *et al.* (2005a)
COMT	22q11	Regulation of extracellular dopamine levels in prefrontal cortex	Egan *et al.* (2001), Shifman *et al.* (2002), Paterlini *et al.* (2005)
RGS4	1q23	Regulator of signal transduction via dopamine, metabotropic glutamate, and muscarinic receptors	Chowdari *et al.* (2002)
PPP3CC	8p21	Subunit-specific function unknown; potential involvement in synaptic plasticity and D1 receptor signaling	Gerber *et al.* (2003)
AKT1	14q32	Multifunctional; possible involvement in D2 and $GABA_B$ receptor signaling	Emamian *et al.* (2004)

in schizophrenia, although relatively low, is ∼12–80 times the estimated general population rate (Karayiorgou *et al.*, 1995). Individual genes from this locus have been examined in systematic fine-mapping efforts (Karayiorgou and Gogos, 2004). LD analysis using 72 SNPs in family samples identified an over-transmission of a haplotypic variant located at the 3′ end of the proline dehydrogenase (*PRODH*) gene (Liu *et al.*, 2002a,b). This finding was replicated in 3 independent family samples, including a very large collection of 528 families from China (Li *et al.*, 2004a) and 274 families of Ashkenazi Jewish origin (Fallin *et al.*, 2005). One negative family study has also been reported (Williams *et al.*, 2003a). In addition, 3′ end variants of the gene were also identified as a risk factor for development of psychotic symptoms during adolescence in children with 22q11 microdeletions (Gothelf *et al.*, 2005). The implicated variants are consistently located at the 3′ end of the gene, but their functional consequences are still unknown. However, the Liu *et al.* (2002a) study identified additional rare variants of the *PRODH* gene, which are present either exclusively or in higher frequencies in schizophrenic patients, are generated through gene conversion from a nearby pseudogene (Liu *et al.*, 2002a) and affect highly conserved amino acids leading to drastic reductions in enzymatic activity (Bender *et al.*, 2005). The same variants were described in schizophrenic patients in an independent study, which also identified a small deletion encompassing the *PRODH* gene (and its neighboring gene *DGCR6)* in a schizophrenic patient (Jacquet *et al.*, 2002). PRODH encodes an enzyme that metabolizes L-proline, a putative neuromodulatory amino acid that could directly influence glutamatergic transmission, which is believed to play a central role in the pathophysiology of schizophrenia (Paterlini *et al.*, 2005). A mutation in the mouse orthologue of the human *PRODH* gene in the Pro/Re hyperprolinemic mouse strain has been described (Gogos *et al.*, 1999). These mice demonstrate an increased neurotransmitter release and abnormal plasticity at glutamatergic synapses, as well as distinct abnormalities in dopamine turnover and signaling in the frontal cortex (Paterlini *et al.*, 2005) reminiscent of schizophrenia in humans.

B. Dystrobrevin-Binding Protein 1

Fine-mapping efforts undertaken as a follow-up to evidence for linkage on chromosome 6p24–22 in a sample of Irish families, led to identification of dystrobrevin-binding protein 1 (*DTNBP1*) gene (dysbindin; Straub *et al.*, 2002) as a schizophrenia candidate gene. Several replication studies have been reported but most replication samples used ($N = 9$) were case-control samples (Funke *et al.*, 2004; Morris *et al.*, 2003a; Numakawa *et al.*, 2004; Van Den Bogaert *et al.*, 2003; Williams *et al.*, 2004). Replication of this association has also been attempted in seven family samples, with replications observed in five of them (Fallin *et al.*, 2005;

Hall *et al.*, 2004; Kirov *et al.*, 2004; Schwab *et al.*, 2003; Tang *et al.*, 2003). In the positive studies, there are inconsistencies among the implicated alleles or haplotypes. These inconsistencies may be a product of population stratification or multiple testing. Alternatively, they could be explained by presence of distinct variations affecting different functional elements within the gene that have emerged independently on a more recent ancestral background. In addition, as this locus has not been extensively analyzed outside the confines of the *DTNBP1* gene, the possibility of a neighboring gene as a sole or partial source of the association signal cannot be excluded. Initial expression and functional studies provide some additional support for a role of *DTNBP1* in schizophrenia. *DTNBP1* has a widespread distribution in the brain, including expression in pyramidal neurons in the hippocampus and dorsal lateral prefrontal cortex (DLPFC). *DTNBP1* expression appears to be decreased in schizophrenia in both DLPFC and excitatory pathways of hippocampus (Talbot *et al.*, 2004; Weickert *et al.*, 2004). Interestingly, a substantial fraction of DTNBP1 is presynaptically localized, and preliminary *in vitro* evidence suggests that knockdown of endogenous dysbindin protein results in the reduction of glutamate release, suggesting that dysbindin might influence exocytotic glutamate release (Numakawa *et al.*, 2004). *DTNBP1* is a member of the biogenesis of lysosome-related organelles complex (BLOC; Li *et al.*, 2003), as well as the dystrophin protein complex (DPC; Benson *et al.*, 2001).

C. Neuregulin 1

A broad region on chromosome 8p12-21 has been implicated in schizophrenia by multiple linkage studies, including a study of 33 extended Icelandic families. Fine mapping in the same set of families detected an association between schizophrenia and several haplotypes at the neuregulin 1 (*NRG1)* locus. A core haplotype at the 5′ end of the gene comprising several markers within a 290-kb block of LD showed highly significant association with schizophrenia (Stefansson *et al.*, 2002). Several replication studies have been reported. Eight of the replication samples used were case-control samples (Corvin *et al.*, 2004; Iwata *et al.*, 2004; Li *et al.*, 2004b; Petryshen *et al.*, 2005b; Stefansson *et al.*, 2003; Tang *et al.*, 2004; Williams *et al.*, 2003b; Zhao *et al.*, 2004) and eight family samples. In the family samples, less than half show some evidence for association, but often with haplotypes other than the one originally described (Duan *et al.*, 2005a; Fallin *et al.*, 2005; Hall *et al.*, 2004; Li *et al.*, 2004b; Petryshen *et al.*, 2005b; Thiselton *et al.*, 2004; Yang *et al.*, 2003; Zhao *et al.*, 2004). Dramatic differences in the frequency of haplotypes reported between different samples (ranging from 1% to 10%; Li *et al.*, 2004b; Zhao *et al.*, 2004) could indicate either substantial heterogeneity in the LD structure across the *NRG1* locus or presence of multiple risk

alleles. In the absence of any functional significance for any of the implicated haplotypes, it is difficult to interpret further the genetic data that is published at the time of this writing. The *NRG1* gene encodes a well-characterized protein involved in a wide variety of neuronal functions, ranging from neuronal survival to myelination and synaptic plasticity (Corfas *et al.*, 2004).

D. G72

A strong and consistent linkage signal for both schizophrenia and bipolar disorder has been identified on chromosome 13q32–34 (Blouin *et al.*, 1998; Detera-Wadleigh *et al.*, 1999) and has prompted fine-mapping efforts. Significant association with schizophrenia was described for several SNPs and haplotypes at the *G72* locus in a French-Canadian case-control sample. The association for two SNPs was replicated in a Russian case-control cohort (Chumakov *et al.*, 2002). Consistent with the linkage studies results, an association between variants at the *G72* locus and bipolar disorder has also been described (Hattori *et al.*, 2003). *G72* association with schizophrenia has been observed in several additional samples including case-control (Korostishevsky *et al.*, 2004; Schumacher *et al.*, 2004; Wang *et al.*, 2004) and family-based samples (Addington *et al.*, 2004; Zou *et al.*, 2005) with evidence for allelic heterogeneity. Negative studies have also been reported (Mulle *et al.*, 2005). Expression and functional studies suggested a potential interaction of *G72* with D-amino acid oxidase that modulates its enzymatic activity and thus could indirectly affect glutamatergic signaling (Chumakov *et al.*, 2002; Mothet *et al.*, 2000). However, this interaction remains to be demonstrated *in vivo*.

E. Disrupted in Schizophrenia 1

Disrupted in schizophrenia 1 (*DISC1*) is one of two genes isolated from a chromosome 1q42 translocation breakpoint previously shown to segregate with psychopathology in a large Scottish family. The other gene is *DISC2* and is a noncoding, presumably regulatory RNA (Millar *et al.*, 2000). *DISC1* was originally described 5 years ago, but interest in it was renewed only recently when large-scale linkage (Ekelund *et al.*, 2001, 2004) and follow-up systematic association studies in Finnish families identified *DISC1* as a positional candidate from the 1q42 locus (Hennah *et al.*, 2003). *DISC1* association with schizophrenia has been observed in some additional samples with evidence for allelic heterogeneity, but negative studies have also been reported (Fallin *et al.*, 2005; Hennah *et al.*, 2003; Hodgkinson *et al.*, 2004). *DISC1* association with schizophrenia-related endophenotypes has been also reported. In one preliminary imaging study, variation in the *DISC1* gene

was associated with altered hippocampal structure and function in healthy subjects (Callicott *et al.*, 2005). An independent study implicated *DISC1* variation in visual working memory performance (Hennah *et al.*, 2005). A family afflicted with schizophrenia and schizoaffective disorder was shown to segregate a rare frameshift variant of the gene (Sachs *et al.*, 2005). *DISC1* is a complex gene with poorly understood involvement in development and synaptic plasticity. It is associated with numerous cytoskeletal proteins, and it could be involved in centrosomal and microtubule function, cell migration, neurite outgrowth, membrane trafficking of receptors, mitochondrial function, and possibly phosphodiesterase signaling (Morris *et al.*, 2003b).

F. Carboxyl-Terminal PDZ Ligand of Neuronal Nitric Oxide Synthase

Brzustowicz *et al.* (2000) have previously reported evidence for linkage at 1q22. Fine mapping using 14 microsatellite markers and 15 SNPs from a subregion of the linkage locus (Brzustowicz *et al.*, 2004) produced nominally significant evidence of LD between schizophrenia and a subset of markers located within the genomic region of carboxyl-terminal PDZ ligand of neuronal nitric oxide synthase (*CAPON*). Abnormal expression pattern of this gene was observed in brains from individuals with schizophrenia (Xu *et al.*, 2005) making it a prime positional candidate from the schizophrenia susceptibility locus on 1q22. Two case-control replication studies (one positive and one negative) have been reported (Puri *et al.*, 2005; Zheng *et al.*, 2005). CAPON is involved in NMDA receptor-coupled nitric oxide signaling (Jaffrey *et al.*, 1998).

G. ZDHHC8

Involvement of this gene was identified in the same LD screen of the 22q11 locus that led to the discovery of the *PRODH*-schizophrenia association (Liu *et al.*, 2002a,b). It was shown that one of the *ZDHHC8* risk alleles (at SNP rs175174), located in intron 4, affects the ratio of an intron 4-containing unspliced form (that encodes a putative truncated form of the protein) over the fully spliced active form (Mukai *et al.*, 2004). The presence of the risk allele rs175174-A results in the production of relatively higher levels (~25%) of the unspliced inactive form (Mukai *et al.*, 2004). Other variants of the gene (affecting distinct aspects of its complex splicing or its expression level) might modulate the disease risk in other nondeleted patient samples. One positive and one negative family-based study of nondeleted patients have been reported so far (Chen *et al.*, 2004a; Glaser *et al.*, 2005). The general involvement of this gene in schizophrenia awaits analysis of additional family samples, but the effect of the gene is predicted to be much stronger in individuals with 22q11 deletions and schizophrenia, where a 50%

(or ~65% when the nondeleted allele carries the risk SNP rs175174 variant) decrease in ZDHHC8 activity levels is predicted. *ZDHHC8* is predicted to encode a transmembrane palmitoyltransferase that modifies PSD-95 among other targets and could play an important role in excitatory synaptic transmission (el-Husseini and Bredt, 2002).

H. Trace Amine Receptor 6

A broad area on chromosome 6q (6q13-26) has been implicated in schizophrenia in linkage studies using European ancestry and African-American schizophrenia pedigrees (Levinson *et al.*, 2000). Two-stage SNP-based fine-mapping efforts focusing on band q23.2 identified trace amine receptor 6 (*TAAR6*) as a prime positional candidate (Duan *et al.*, 2004) for the schizophrenia susceptibility locus on 6q23.2. Two negative replication studies have been reported (Duan *et al.*, 2005b; Ikeda *et al.*, 2005). An independent study implicated the trace amine receptor genes at 6q23.2 in susceptibility to bipolar disorder (Abou Jamra *et al.*, 2005). *TAAR6* is a GPCR widely expressed in the brain (Borowsky *et al.*, 2001).

I. Epsin 4

Chromosome 5q33 is a region that has previously shown strong evidence of linkage to schizophrenia in four independent linkage studies. Four adjacent markers (and associated haplotypes) at the 5′ end of the *Epsin 4* (*EPN4*) gene, which is located in this region, showed significant evidence of LD with schizophrenia in a case-control fine-mapping study (Pimm *et al.*, 2005). The *Epsin 4* gene encodes the clathrin-associated protein enthoprotin, which has a role in transport and stability of neurotransmitter vesicles at the synapses and within neurons. No replication studies (especially family based) have been reported yet.

J. $(GABA)_A$ Receptor Subunit Gene Cluster

Chromosome 5q31–35 was implicated in Portuguese schizophrenia families (Sklar *et al.*, 2004) and was supported by subsequent meta-analysis. A group of γ-aminobutyric acid $(GABA)_A$ receptor subunit genes (*GABRA1, GABRA6, GABRB2, GABRG2*, and *GABRP*) that map within this linkage peak were examined in Portuguese patients, and associations with SNPs and haplotypes in *GABRA1, GABRP*, and *GABRA6* were detected (Petryshen *et al.*, 2005a). The *GABRA1* and *GABRP* findings were replicated in an independent German family-based sample (Petryshen *et al.*, 2005a). These genes are plausible candidates based on prior evidence for GABA system involvement in schizophrenia (Lewis *et al.*, 2005).

III. Other Candidate Genes

The candidacy of the genes described in this section is based on convergent genetic and biological evidence rather than on positional cloning. Interestingly, many of these genes are located in the general vicinity of linkage signals. Although far from proven, the recurrent observation of clustering of candidate susceptibility genes could indicate that more than one gene could contribute to at least some of the linkage signals in psychiatric disorders.

A. Catechol-*O*-Methyltransferase

The gene is located in the 22q11 region between the *PRODH* and *ZDHHC8* genes. In addition to being a positional candidate gene, catechol-*O*-methyltransferase (*COMT*) is also an attractive functional candidate gene since it is involved in the breakdown of dopamine. One variant in particular, in codon 158 that affects enzymatic activity depending on presence of Val (high activity) or Met (low activity), has been examined extensively in studies testing directly for association with schizophrenia. It has been proposed that the high-activity Val allele increases the risk for schizophrenia but the genetic association results are equivocal (Fan *et al.*, 2005; Glatt *et al.*, 2003; Lohmueller *et al.*, 2003; Munafo *et al.*, 2005; Shifman *et al.*, 2002; Tsai *et al.*, 2006; Williams *et al.*, 2005). The same allele was shown in some studies to impair executive function, which is affected in schizophrenic patients (Egan *et al.*, 2001; Ho *et al.*, 2005). Studies in animal models, however, suggested that low activity of this enzyme could be a risk factor for schizophrenia by failing to buffer the effect of other primary mutations that affect dopamine turnover and signaling in the cortex (Paterlini *et al.*, 2005). This prediction was supported by the results of a longitudinal follow-up study of children with 22q11 microdeletions, which revealed that the low-activity form of the enzyme (Met158) is a risk factor for decline in prefrontal cortical volume and cognition, as well as for the consequent development of psychotic symptoms during adolescence, in these children (Gothelf *et al.*, 2005). Therefore, the contribution of *COMT* to schizophrenia, in general, is likely to be complex.

B. Regulator of G-Protein Signaling 4

The gene maps to 1q21–22, 0.7 Mb from *CAPON* (see Section II). Regulator of G-protein signaling 4 (*RGS4*) was initially identified as the only transcript (out of 7800 sampled by Mirnics *et al.*, 2000) consistently reduced in the DLPFC of individuals with schizophrenia. Subsequently, Chowdari *et al.* (2002) genotyped 13 SNPs across a 300-kb segment spanning the gene in several independent

datasets and found weak evidence for association with schizophrenia in each of the samples within a haplotype block stretching from intron 1 to several kb upstream of the transcription start site. However, the pattern of allelic association was not consistent among samples. Independent replication efforts have been reported including both positive and negative studies (Chen *et al.*, 2004b; Fallin *et al.*, 2005; Sobell *et al.*, 2005; Zhang *et al.*, 2005). *RGS4* is one of 19 human *RGS* transcripts and is abundant in the cerebral cortex (Larminie *et al.*, 2004). *RGS4* encodes for a GTPase activator, which desensitizes Gi/Go and Gq, thus negatively modulating G-protein-mediated signaling via dopamine, metabotropic glutamate, and muscarinic receptors (Ross and Wilkie, 2000).

C. Calcineurin Gamma Catalytic Subunit

PPP3CC which encodes the calcineurin gamma catalytic subunit is located at 8p21.3, 10 Mb from *NRG1*, but adjacent to previously described linkage signals (Gerber *et al.*, 2003). Forebrain-specific calcineurin knockout mice were reported to have a spectrum of behavioral abnormalities related to altered behaviors observed in schizophrenia patients (Miyakawa *et al.*, 2003) supporting the proposal that alterations in calcineurin signaling contribute to schizophrenia pathogenesis. In further support of this proposal, *PPP3CC* was found to be downregulated in the hippocampus of individuals with schizophrenia (Eastwood, 2005). The genetic association was not replicated, however, in a sample of Ashkenazi Jewish nuclear families (Fallin *et al.*, 2005). Calcineurin is a multifunctional calcium-dependent serine/threonine phosphatase, known to be centrally involved in many aspects of synaptic plasticity. It has particular roles in glutamate and dopamine signaling and their interactions, including regulation of DARPP32, a molecular node of convergence between dopamine receptor 1 and NMDA receptor signaling pathways (Miyakawa *et al.*, 2003; Winder and Sweatt, 2001).

D. AKT1

AKT-GSK3beta signaling is a target of lithium and as such has been implicated in the pathogenesis of mood disorders. Evidence was provided that this signaling pathway also has a role in schizophrenia (Emamian *et al.*, 2004), including convergent evidence for a decrease in AKT1 protein levels and levels of substrate phosphorylation in the peripheral lymphocytes and brains of individuals with schizophrenia; a significant association between schizophrenia and an *AKT1* haplotype associated with lower AKT1 protein levels; and a greater sensitivity to the sensorimotor gating-disruptive effect of amphetamine, conferred by AKT1 deficiency. The genetic association has been confirmed thus far in two

independent populations, namely in a combined sample of ~1000 cases and 1000 controls from Japan (Ikeda *et al.*, 2004; Ohtsuki *et al.*, 2004) and in a sample of European sib-pair families (Schwab *et al.*, 2005). Dopamine plays an important role in the etiology of schizophrenia, and despite claims that most behavioral actions of DA are associated with the modulation of adenylate cyclase and PKA activity (Greengard, 2001), investigations have uncovered that stimulation of D2 class receptors also results in a adenosine 3′,5′-monophosphate (cAMP)-independent dephosphorylation/inactivation of Akt (Beaulieu *et al.*, 2004) associated with the expression of DA-dependent behaviors (Beaulieu *et al.*, 2004; Emamian *et al.*, 2004). These novel results suggest a model in which both cAMP-dependent and cAMP-independent events play important and perhaps cooperative functions mediate schizophrenia-related DA actions. Consistent with this model, administration of haloperidol or the selective D2 class receptor antagonist raclopride has been shown to prevent the regulation of Akt by DA or enhance Akt phosphorylation in animal models (Beaulieu *et al.*, 2004; Emamian *et al.*, 2004) and thus, in principle, could compensate for an impaired function of this signaling pathway in schizophrenia. Moreover, two other drugs used in the management of psychosis, lithium, and clozapine also act as enhancers of Akt signaling *in vivo* (Beaulieu *et al.*, 2004) or *in vitro* (Chalecka-Franaszek and Chuang, 1999; Kang *et al.*, 2004). Akt signaling has also been implicated in GABAergic transmission (Wang *et al.*, 2003) and outside the CNS has been implicated in the regulation of multiple biological processes ranging from glycogenesis to embryonic development, apoptosis, and cell proliferation (Scheid and Woodgett, 2001).

IV. Areas of Caution in the Interpretation and Generalization of Genetic Findings

A combination of criteria that include the degree of statistical significance, the reproducibility of the associations in independent samples, the identification of independent rare risk alleles, and the consistent findings from animal model studies and endophenotype-based studies in humans need to be considered in assessing the degree of confidence assigned to each of the findings outlined in the sections above. On the basis of these criteria, support for at least some of the findings described above (such as *PRODH, DTNBP1, NRG1, G72, DISC1*, or *COMT*) appears to be quite strong. However, it should be emphasized that, even for the stronger findings, it is too early to draw firm conclusions about their generalization among different samples and populations primarily due to uncertainties pertaining to the extent of coverage of the implicated loci, consistency of the risk allele or risk haplotype across studies, the structure of the samples used in the original and replication studies, publication bias against negative reports, and supporting biological data. Because of these issues, claims of "replication" should

be taken with caution and the reader is encouraged to undertake a careful analysis of the properties of the employed samples and the methods used in order to determine the validity of such claims.

What are the general areas of caution in the interpretation of these findings? In general, the statistical burden of proof is lower for genes identified through systematic follow-up of linkage signals compared to genes picked in essentially random fashion irrespective of their location relative to linkage signals (this is discussed in detail in Freimer and Sabatti, 2004). Another important issue of concern regards the structure of the tested replication samples.

It is becoming increasingly clear that unreliable results may be obtained when allele frequencies differ notably among subpopulations not represented equally between cases and controls (Campbell *et al.*, 2005). Therefore, the possibility that original or replication studies using case-control samples are false positives (or negatives) is a major source of concern. This issue is relevant to all common, complex disorders, but it is likely to be more pronounced in genetic studies of psychiatric disorders, which are confounded by a larger degree of phenotypic heterogeneity. Even more alarmingly, several of the original or "replication" samples have been used repeatedly in genetic association studies making the issue of multiple testing corrections highly relevant. These are not merely theoretical considerations as they can lead to striking inconsistencies among variant alleles and haplotypes implicated in various replication studies (inconsistencies are sometimes observed even in more reliable family-based samples and can be explained in some instances by presence of distinct variations affecting different functional elements within the gene that have emerged independently on a more recent ancestral background). Publication bias almost certainly affects the level of confidence ascribed to any given susceptibility gene, primarily because negative studies are more likely to accumulate with considerable delay or not at all (negative studies are less likely to be submitted for publication and when they are submitted they are less likely to be published in the same journals where the original discovery was reported).

V. Future Directions of the Genetic Research: Advancing Our Understanding of How the Specific Genetic Factors Contribute Biologically to the Disease Process

The recent gene discovery studies promise to provide researchers with important new clues regarding the genetic causes of schizophrenia. As additional genes are identified through linkage or genome-wide association studies (which are now starting to be implemented) a central goal of future research will be to understand the functional implications and interactions of the susceptibility genes and their variants in the context of schizophrenia.

In the absence of well-defined and fully penetrant mutations, similar to the ones found in Mendelian disorders, it is important to balance human genetic and hard biological evidence against the need for timely identification of targets and improvements in therapy. Genetic studies of endophenotypes (Egan *et al.*, 2001) (provided they are designed to avoid all the pitfalls described above, which are associated with genetic studies of the clinical syndrome) and most importantly hard biological data from animal model studies promises to advance our understanding of the disease pathophysiology in the next few years.

A. Animal Models

Identification of susceptibility genes will permit incisive studies to illuminate the physiological and biochemical etiology of the disease by examining the gene products in the context of a model organism and their impact on the development of the disorder. Such studies could also provide a critical resource for testing new mechanism-based candidate therapies. It remains a challenge, however, to define the optimal means to harness such model organisms in investigative strategies designed to understand and manipulate candidate factors predisposing to schizophrenia, a uniquely human disorder. For example, generation of *bona fide* mouse models for most psychiatric disorders is highly unlikely due to constraints imposed by the complex polygenic nature of human psychiatric disorders, by the magnitude and pattern of change during hominid brain evolution and by uncertainty regarding the clinical features of the human syndromes. On the other hand, mouse models of "susceptibility genes" identified through forward genetic studies in humans hold tremendous promise in understanding the function of the genes in the context of simple cellular pathways or even at the level of simple neural circuits and behavior.

Even in this context, there are several important factors that need to be considered in generating such models and in designing and interpreting their analysis. Possibly the most important consideration concerns the nature of the susceptibility allele. For example, it is critical to know whether the risk allele constitutes a hypomorph or a gain of function to predict whether a mouse knockout allele can model it accurately. A related consideration concerns potential broad expression and pleiotropic effects of particular susceptibility genes. Several of the strong candidate genes (i.e., *NRG1)* appear to participate in virtually all aspects of brain development, maturation, and function and modulate signaling through a large number of neurotransmitter receptors (Corfas *et al.*, 2004). Given this complexity, when modeling such genes using, for example, mouse knockout approaches, one must consider carefully which of a large number of alternative phenotypes might provide a critical link between the genetic risk variant and susceptibility to schizophrenia. Therefore, one very important goal

of human genetic research will be: (1) to define as accurately as possible the risk alleles or haplotypes and (2) to decipher the functional implications of the risk alleles or haplotypes (Pastinen and Hudson, 2004) in order to model them in mice as closely as possible. Given our present limited understanding of the functional impact of human genetic variation, accurate mouse models of risk alleles have been reported for only a small number of schizophrenia susceptibility genes (Huotari *et al.*, 2002; Paterlini *et al.*, 2005; Paylor *et al.*, 2001). Provided that reliable mouse models are available, mechanistic insights into the mode of contribution of these genes, as well as their interactions can be obtained through a heuristic progression starting from the molecular level to the cellular and synaptic level to the systems level and culminating at the behavioral level.

B. Genetic Interactions

The genetic complexity of common psychiatric disorders has been repeatedly inferred from the pattern of inheritance and the inability of the research community to identify consistent linkage signals. It is believed that genetic interactions among susceptibility genes (especially epistasis) lie at the core of this complexity. Generally speaking, epistasis is a phenomenon whereby the effects of a given gene on a biological trait are masked or enhanced by one or more other genes. It has been speculated that this type of genetic buffering leads to phenotypes that are stable in the presence of mutations (Moore, 2005). It has also been argued that for a phenotype to be buffered against the effects of mutations, it must have an underlying genetic architecture that is composed of networks of genes that are redundant and robust (Moore, 2005). As a result, substantial effects on the phenotype are observed only when there are multiple mutational hits to the gene network.

The biological basis of these epistatic interactions remains elusive in psychiatric disorders, but two studies provided some important relevant insights (Millar *et al.*, 2005; Paterlini *et al.*, 2005). One study, using animal models, demonstrated a clear epistatic interaction between the *prodh* and *comt* genes at the level of transcription and behavior that is likely to represent a *comt*-modulated homeostatic response to abnormal dopaminergic signaling in the frontal cortex that emerges as a result of prodh deficiency (Paterlini *et al.*, 2005). This is an intriguing finding, because dopaminergic dysregulation in schizophrenia is well established, based primarily on the therapeutic effect of dopamine receptor antagonists (Seeman, 1987). Moreover, based on clinical and preclinical observations, it has been suggested that this dopaminergic dysregulation emerges as a secondary result of other primary deficits, including impaired glutamate transmission. In any case, it is conceivable that similar patterns of genetic interactions that involve impaired synaptic function and impaired homeostasis or compensation

(genomic buffering) might also account for the epistatic component of the genetic risk of psychiatric disorders in general.

A second study used the two-hybrid system to identify a molecular interaction between the DISC1 protein and phosphodiesterase 4B (PDE4B; Millar *et al.*, 2005). Although the gene encoding for PDE4B is not included in the list of strong candidate genes outlined above, the authors showed it is disrupted by a balanced translocation in a subject diagnosed with schizophrenia and a relative with chronic psychiatric illness. The PDEs inactivate cAMP, a second messenger implicated in learning, memory, and mood. It was shown that DISC1 interacts with the UCR2 domain of PDE4B and that elevation of cellular cAMP leads to dissociation of PDE4B from DISC1 and an increase in PDE4B activity. The authors proposed a genetic interaction model whereby DISC1 sequesters PDE4B in resting cells and releases it in an activated state in response to elevated cAMP.

Methods of analysis designed to probe epistasis are clearly of growing importance in the genetic dissection of complex disorders and currently a variety of methods exist to detect or control for the presence of epistasis. These methods are limited by the need for large sample sizes that ensure adequate power to detect gene interactions. Moreover, direct biological inference from the results of statistical tests is very difficult because statistical interaction does not necessarily imply interaction at the biological level (Thompson, 1991). In all, the degree to which statistical modeling can provide insights into the underlying disease mechanisms is likely to be limited, and might require prior knowledge of the underlying etiology. The question of true biological interaction remains of extreme importance in the field of complex psychiatric genetics, but might ultimately be better answered primarily via a combination of molecular and animal model-based approaches.

C. Understanding Disease Pathophysiology

There are two major pharmacological hypotheses regarding the pathophysiology of schizophrenia—the dopaminergic and glutamatergic hypotheses. The dopaminergic hypothesis is based primarily on the observation that all drugs with efficacy in treating symptoms of schizophrenia share the property of dopamine D2 receptor antagonism and also on the fact that indirect dopaminergic agonists, such as cocaine and amphetamine, have psychotomimetic properties (Seeman, 1987). The glutamatergic hypothesis arose from the finding that phencyclidine (PCP), a potent psychotomimetic drug, is an antagonist of the NMDA receptor, and posits that a major underlying cause of schizophrenia is abnormal glutamatergic transmission, particularly in the prefrontal cortex, limbic areas, and striatum (Coyle, 1996). Since the glutamatergic and dopaminergic systems

are known to have complex interactions, the two hypotheses are not incompatible, and it is likely that primary changes in one system would lead to associated alterations in the other.

Although efforts for synthesis of the initial genetic findings in the context of specific neurotransmitter systems have been reported (Moghaddam, 2003), it might be premature at this point to claim that the existing genetic data support the critical involvement of one neurotransmitter system over another. The genes outlined in this review article are involved in several neurotransmitter systems. For some of them (such as *PRODH, G72, DTNBP1, NRG1, ZDHHC8, CAPON*), there is variable degree of experimental evidence that they are involved in excitatory glutamatergic pathways. For other genes, there is clear evidence of involvement in dopamine (*COMT, AKT1*), GABA (5q *GABA* receptor cluster, *AKT1*), or trace amine signaling (*TAAR6*). However, even for genes that primarily act via disruption of excitatory synaptic function, the final effect could be mediated by abnormal dopamine signaling (Paterlini *et al.*, 2005; see previous paragraph). Finally, many of the genes (such as *NRG1, DISC1, PPP3CC, RGS4, AKT1*) appear to have pleiotropic effects that are not restricted to a particular type of synaptic transmission and involve several aspects of neuronal biology. While it is too early to determine from the existing genetic evidence how neurotransmitter systems might be primarily affected in the disease, identification of additional genes and further functional studies will help elucidate this issue. However, it is equally likely that the final cumulative effect of the risk variants will emerge from, and be determined by, the pattern of expression, as well as the pattern of interaction among these genes, and it could be restricted to specific brain regions, specific cell types, or both, rather than specific neurotransmitter systems. This regional or cellular selectivity could underlie the differences and commonalities among common psychiatric disorders, as well as their distinction from other common and serious CNS conditions, such as mental retardation, or epilepsy that employ common neurotransmitter systems.

D. Mechanism-Based Therapies

It is common place to state that understanding of the function and the interactions among individual susceptibility elements could eventually lead to design of highly effective targeted therapies for patients with specific genetic predisposition with fewer side effects and more positive long-term disease outcomes. However, optimism should perhaps be tempered by experience gained from study of simple genetic conditions where knowledge of the genes and protein alterations is often available, and yet it has proven highly challenging to translate this detailed knowledge into creation of therapies. It is conceivable that despite their more complex etiology, multifactorial disorders like schizophrenia may be

more amenable to mechanism-based therapeutic intervention. So far, it appears that genetic alterations contributing to schizophrenia consist largely of common but relatively subtle variations presumably affecting transcript expression or processing and in some cases protein function. The disease risk associated with such variations is usually very low, but because the risk alleles are so common, a low disease risk corresponds to a large population attributable risk (which means that if the population were monomorphic for the nonrisk allele, the prevalence of the disease would be considerably lower). Directed therapies, therefore, might only need to provide relatively modest modulation of appropriate molecular targets to reach an effective threshold in a large fraction of patients, in contrast to simple conditions, in which compensation for more pronounced functional alterations might be required.

Acknowledgments

I thank David Gerber and Maria Karayiorgou for helpful insights and comments on the chapter and members of my laboratory for discussions. My research is supported by grants from the McKnight Endowment Fund for Brain Disorders, the EJLB Foundation, the New York City Council Speaker's Fund for Biomedical Research, and NARSAD.

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INDEX

D

G

H

N

Q

T

V

W

X

Y

Z

CONTENTS OF RECENT VOLUMES

Volume 37

Volume 38

Volume 39

Volume 40

Volume 41

Volume 42

Volume 43

Volume 44

Volume 45

Volume 46

Volume 47

Volume 48

Volume 49

Volume 50

Volume 50

Volume 52

Volume 53

Volume 54

Volume 55

Volume 56

Volume 57

Volume 58

Volume 59

Volume 60

Volume 61

Volume 62

Volume 63

Volume 64

Volume 65

Volume 66

Volume 67

Volume 68

Volume 69

Volume 70

Volume 71

Volume 72

Volume 73

Volume 74

Volume 75

Volume 76