Handbook of Medical Psychiatry

edited by

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Medical Psychiatry

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Series Introduction

In the late 1950s and early 1960s many of the senior professors of psychiatry, including those who were psychoanalysts, also had extensive training in neurology. Some departments, including the one in which I trained, were departments of psychiatry and neurology. To be certified as a psychiatrist, one of the three patients you examined and were questioned about was a patient with primary neurological disease. We were expected to know how to recognize seizure spindles in an EEG, and to be able to point out the anatomy and pathology visible in brain slices. The neurology candidates were similarly examined and questioned in psychiatry. Many practitioners did a bit of both: for example, the senior neurologist in the department in which I trained made his own diagnoses of depression, and administered ECT to the patient in his office.

Outside the "black box" of the skull, our ties to the rest of medicine were not as strong. This was true despite the attempts to promote both concepts of "psychosomatic" medicine and humane care. Unfortunately, neither the concepts nor the data were strong enough to carry the day. More recently, however, the development of new technologies, such as imaging and explication of the genetic code, has resulted in an explosion of knowledge about human biology and pathology. Newer findings have begun to break down the barriers between psychiatry and neurology, and between our understanding of behavioral disorders and the rest of medicine. While I do not believe that we will ever be able to do without a psychology in psychiatry, it is also increasingly clear that psychiatry cannot function without understanding the biology of the brain.

Drs. Soares and Gershon have done an excellent job in bringing together a group of outstanding contributors who bring this new understanding to our field. They have presented the complex material clearly and comprehensively, making it easier to master—a necessary task if we are to continue to help our patients.

William A. Frosch

Foreword

With the publication of the *Handbook of Medical Psychiatry*, Drs. Soares and Gershon have recaptured the traditions of psychiatry over the past century, added the impressive technical capacities of the field in the last decade, and created an important educational resource for this new century. The deceptively simple title of the book belies the scope and depth of the volume. The chapters are organized, in part, by major diagnostic categories (e.g., mood disorders, schizophrenia, and related disorders, etc.), but also by significant crosscutting issues such as research methods and psychopharmacology.

Within this rich composite of important theoretical and practical information the editors have integrated critical themes of modern psychiatry such as:

Classification/nosology. DSM-III and -IV and ICD-10 are clearly interim steps in the development of disease-based classification systems for psychiatry. However, movement from phenomenology to etiolopathogenesis will have many steps along the way. This book examines fundamental issues in diagnosis across the range of psychiatric disorders and will help prepare psychiatrists to better understand strategies to move along that path.

Mechanisms. Ultimately, a disease-based classification will require the elucidation of the basic mechanisms underlying mental disorders. The book presents, in a provocative manner, current leads in neurochemistry, neurocircuitry, molecular biology, and genetics, among other fields.

Tools. Realizing that a more comprehensive understanding of basic mechanisms will evolve over decades, the authors have provided the reader with an understanding of the remarkable tools now available in imaging, molecular biology, and genetics. These new technologies enable researchers to open the "black boxes" of the brain, the cell, and the gene. Understanding how these tools are applied and getting a taste of current findings will enhance the reader's ability to become educated consumers of the barrage of information that is, and will be, emanating from the tremendous growth of research activity in psychiatry.

Evidence. Over the past decades, the values of psychiatry have become more and more closely aligned with the values of science. As such, the ability to interpret and evaluate scientific evidence and augment it with clinical insights is a critical skill. It is a skill that must be not only learned during medical school and

residency but also continuously exercised over the course of every psychiatrist's career. The authors have effectively captured the essence of that task in this volume. Readers who engage this important and challenging material will be revitalizing those skills and also preparing themselves for the future. Impressively, Drs. Soares and Gershon have enlisted the talents of many of the world's leading clinicians and scientists in this ambitious work. Even more importantly, they have tapped some of the most promising younger psychiatrists who will be major contributors in expanding our understanding of psychiatric disorders in the future.

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Preface

Over the past few decades, increasing emphasis has been given to the study of brain mechanisms that may be dysfunctional in neuropsychiatric illnesses. In recent years, new methodologies from various disciplines in the clinical neurosciences have made available substantially improved and more sophisticated tools for studies of causation of these severe illnesses. For instance, we have seen tremendous progress in knowledge from disciplines such as molecular genetics, neuropsychopharmacology, and brain imaging, which has provided unprecedented tools for studies of the human brain and neuropsychiatric illnesses. These efforts have begun to produce important findings and are beginning to contribute to a better understanding of the basic mechanisms involved in these disorders and the mechanisms of actions of treatments for these conditions, and lead to the development of new therapeutic possibilities.

The *Handbook of Medical Psychiatry* summarizes the main advances in the understanding of the basic mechanisms and therapeutics of the major psychiatric illnesses that have taken place in recent years. The format provides easy access to new information in these areas, making the book of significant interest to academicians, researchers, practitioners, students, residents, and trainees in psychiatry, clinical neuroscience, and the mental health professions. We believe this book will be a helpful, comprehensive, and important resource for individuals in psychiatry and related fields.

Jair C. Soares
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Animal Models of Neuropsychiatric Disorders
Challenges for the Future

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I. INTRODUCTION

The major challenges for future animal modeling research primarily involve conceptual and philosophical issues. Despite the fact that there are a variety of animal models available for many psychiatric disorders [1–3] there are still widespread perceptions that (1) one cannot reasonably study human psychiatric disorders in animals, because psychiatric illnesses are inherently human, and (2) there are no animal models of the various psychiatric disorders available.

In medicine in general, animal models are generally accepted as important for research directed at understanding the mechanisms underlying human disease as well as the development of new treatments. In contrast, modeling of mental disorders in experimental animals has often been regarded as "a highly controversial or outright heretical idea" [4]. There is widespread skepticism regarding animal models in psychiatry, with virtually no organized federal programs/initiatives for encouraging and supporting research in this field. When the active opposition of components of the animal rights movement to research with animals is coupled with the above-mentioned lack of understanding of the role of animal models by leaders in the field (who sometimes have backed off encouraging further developments in this field in the face of the animal rights movement), the stage is set for continuing major problems. This in summary represents the major future challenges for the field of animal modeling research.

The above situation is ironical given the role that research utilizing animal models has played in advancing the understanding of psychiatric disorders. As will be discussed in the historical section, the first data-based integrative theories of psychopathology grew largely out of animal research and/or improving treatment approaches. Since psychiatric disorders need to be understood by using a multivariate approach, animal studies, where variables can be controlled, have the potential for permitting the study of both the main effects of single variables and especially their interaction. Such approaches are highly relevant to what has recently been termed the "biopsychosocial" view of human psychopathology [5].

Research with animals has also been critical in broadening our understanding of human development and in providing empirical support for the importance of early experiences for behavioral and neurobiological development. Work with multiple species has documented the central importance of early social attachment systems and has clarified the behavioral and neurobiological variables mediating the development of these attachment systems [6–10]. Such concepts are

by now woven into the fabric of development theory in adult and child psychiatry, and it was experimental animal research that provided the fundamental basis for this knowledge. Furthermore, research with experimental animal systems has documented the devastating and long-term behavioral and neurobiological effects, including effects on brain cytoarchitecture, of never letting such attachment systems develop or of their intermittent disruption at certain developmental stages [11–14].

Studies utilizing animal models have also focused on the interaction between social functioning and neurobiological status and have documented the interactive and reciprocal nature of these relationships in paradigms that would be impossible to implement in human studies [15,16]. In addition, the impact of different types of stress has been extensively explored in animal models and in some instances genetic strains have also been identified or developed which exhibit similarities to clinical syndromes [17,18].

Though there is no perfect animal model with regard to predicting clinical efficacy of pharmacological agents, their use has been critical in the discovery and development of drug treatments [19–22]. There are always false positives and false negatives, but there are experimental paradigms with a high degree of empirical validity. Given a new era of drug discovery and development, there will likely continue to be many challenging issues in this context.

Despite these and many other contributions, acrimonious debates about the validity and/or usefulness of animal models for psychiatric disorders persist. The evaluation of animal models for psychiatric disorders is complex. Unfortunately, there is not yet any single laboratory finding or set of findings for any clinical psychiatric syndrome that one could insist upon as part of the validating criteria. Thus, one largely relies on a combination of behavioral measures and response to known clinically effective agents. Part of the challenge for the future may be to reconsider the validity measures of animal models and to reconceptualize expectations.

There is no ''perfect,'' complete, or comprehensive single animal model for any specific psychiatric disorder. Indeed, there will likely never be an animal model in any field of medicine that is a perfect fit with the human condition; rather the emphasis in the development and study of disease models in animals needs to increasingly focus on specific components of the human illness.

Animal models of diseases in medicine, including psychiatry, need to be understood in a historical and evolutionary perspective and their advantages as well as limitations recognized. Neither overextended cross-species comparisons nor unjustified negativism about animal models seems defensible.

An especially critical challenge in the continuing development and utilization of biobehavioral animal models in psychiatry is their relationship to the molecular neurosciences, including genetics. Given recent advances in the molecular neurosciences relevant to mental disorders, the role of animal models in this context needs to be reconsidered. Failure to do so could lead to an excessively narrow view of animal models or a dismissal of the entire area. Danger signals already exist in this regard. Some contend that, given the new molecular techniques, animal models no longer have a place in psychiatric research. Others have taken the position that since psychiatric illnesses are so difficult to model in animals, we will need to do most of the research on mental disorders in clinical populations [23]. There is also tension between those who think that while one can, with high validity and reliability, measure, for example, receptor functioning in certain brain regions, the measure of behavior in animals lacks comparable scientific precision [24]. Unfortunately, the latter reflects a serious lack of communication between fields because the quantitative assessment of animal behavior is a well-developed science.

With the increasing advances in molecular biology and genetics, functional neuroimaging, and other methods for studying mental disorders, conceptualization of and research on behaviorally based animal models needs to be able to keep pace to maximally enrich psychiatric research. Despite several recent publications about the animal modeling field [25–28], there are many indications that the area remains poorly understood. This paper is an attempt to provide an overview of the past contributions of animal models and to propose some new perspectives that might be helpful in reevaluating the role of animal models in better understanding the major psychiatric illnesses. In an attempt to focus on some fundamental issues and challenges regarding animal models as they relate to neuropsychiatric disorders, a review of available models for each disorder becomes impossible. To attempt such a review would certainly shortchange many important areas, so, rather than attempt this, appropriate references will be provided to articles where such models are discussed.

II. HISTORICAL CONTEXT

Pavlov, often said to have been the originator of research relevant to animal modeling of human psychopathology in general, used clinical terms and experimental techniques that now seem foreign to most clinicians. However, the fact that his work represented one of the first moves away from a strictly correlational method of behavioral analysis to the experimental study of psychopathology is of central importance [29].

Considering Pavlov and other early scientists [30–33], it is difficult to know what conclusions to draw about the early history of the field of experimental psychopathology research. From one standpoint it was not a particularly noteworthy beginning. However, the early pioneers may have been more successful than it appears in developing certain principles that seem to be being rediscovered today, including:

1. Demonstration that psychopathology could be experimentally studied in animals as well as in the strictly correlational studies done previously in humans.
2. Demonstration of the importance of both careful behavioral observations and serendipity. Although most of the early workers did not use the more sophisticated and quantifiable behavioral scoring techniques now available, they were keen observers and literate in their descriptions.
3. The repeated proposal of an interactive model of psychopathology. The role of the temperament of the animals, along with a variety of social and neurobiological variables, was repeatedly stressed in the early literature. The concept of individual variability was part of the early work, and investigation of the sources of such variability continues to be an important area of research.
4. Recognition that there could be a persistent internal response, even after the inducing stimulus was no longer present, a discovery that remains a major contribution to the understanding of a number of forms of psychopathology.
5. Recognition of the importance of unpredictability and uncontrollability of which systematic investigations continue today [5].

III. ETHOLOGICAL CONTEXT

This section touches on some principles of ethology important in evaluating and understanding experimental animal research and a few selected research approaches. Avoidance of misleading clinical labeling based on superficial comparisons across species is critical. However, behavioral profiling in a given species can be done with a degree of precision comparable to other methods in neurobiology. Evolutionary biology principles then need to be understood and applied when it comes to interpretation of these phenomena. Ethology focuses on describing and understanding "animal behavior in the natural habitat and assumes operation of evolutionarily conserved basic plans encoded in the genome. Such basic plans determine behavioral patternings including flexible variants involving learning. Human ethology has emerged as a subdiscipline, including observations of psychiatric patients. The research involved has produced an enormous and varied literature" [34].

Gardner describes this interface as follows:

> Sensitive observers have noted that relationshipless psychiatry seems the objective of much current psychiatric practice augmented by the cost-conscious managed care industry. Such a peculiar objective can stand almost unopposed in part because psychiatry has no basic science other than that limited to drug actions on the one hand and venerable, used but unproven and unphysiological theories of psychotherapy on the other hand. Adopting a perspective that shows human relatedness to other animals (near identity of genome) yet human uniqueness (with a massively larger brain) would underline the importance of people for other people that would augment the psychiatric enterprise. Human bonding and human competition shares much in common with other species, yet has its own flavor likely stemming from the human capacity to use stories in many ways. An important step towards psychiatry as a relationship-focused enterprise might come about if there was an explicit label for it. Sociophysiology could furnish that label to emphasize the importance of weighing the following as equally important while interactive: complex behaviors especially communicative ones, ancient reaction patterns, brain functions, cellular actions and genomic mechanisms [35].

Gardner described modeling as depending upon brain-body factors that the animal and humans possess in common. While behavior patterns may be species specific, "core components shared by related animals are typically embroidered through natural selection to produce modified methods of survival and reproduc-

tion'' [34]. He cites the example of human brains which contrast in size to those of other animals, weighing three times more than brains of surviving large primates or those of human ancestors 3 million years ago. The contrast with other primates especially stems from a massively enlarged neocortex, especially the frontal lobes, with these increases likely stemming from advantages of social functions. Despite differences, the human brain and behavior also show comparability to those of other species through widely shared, conserved features. Gardner contends that this comparability makes continued use of animals important for the study of the pathogenesis, mechanisms, and treatment of mental disease. ''Lack of a sufficient database as yet limits other modeling efforts such as computer simulations, mathematical models, and experimentally induced states in hums, and research on animals remains indispensable'' [34].

Homology and convergence are important ethological concepts which can serve as frameworks for helping to understand comparative cross species behavior.

> Homology means that a common ancestor once possessed a trait now shared by two species. At points in the past, humans shared common ancestors with monkeys, mice, chickens, fish, insects, and single celled organisms, each such forebear more remote in biological history. In contrast, convergent traits are similar features that stem from environmental shaping through natural selection, although basic plan starting points vary. Wings of insects, bats and birds illustrate this. Ancestors of each animal group had not flown so airborne ability evolved separately and the three kinds of wings illustrate convergent evolution on the level of aerial locomotion. As vertebrate upper extremities, however, wings of bats and birds are homologous to each other but not to the wings of insects. If the starting point of the basic plan generalizes to contractile tissue, however, locomotory body extensions of all three achieve homology [34].

There is great excitement with genome projects of species at different phylogenetic levels and importance. The genome seems to contain at least a partial record of the organism's ancestry, and therefore genomic analysis may help determine evolutionary history (homology) which in turn may foster knowledge of proximal neuronal determinants of behavior. However, this is a very complicated area, and simplistic and overly optimistic expectations will likely fail. As Gardner says, at the behavioral level, redundant, multiply determined brain-behavior adaptations complicate inference, and at the DNA level, genetic transformations such as chromosomal crossovers will reduce certainty about genomic hypotheses [34].

IV. DEFINITIONAL/CONCEPTUAL ISSUES

Animal models are experimental paradigms developed in one species for the purpose of studying specific phenomena occurring in another species. By definition they are not the ''real thing.'' There will always be differences and similarities between models and what is being modeled; otherwise it is not a model. Furthermore, there is no single comprehensive animal model for any mental disorder and probably not for any general medical illness. Thus, animal models should be judged primarily by their relevance to specific questions that they are being used to address rather than their scope. They permit the evaluation of selected aspects of human psychopathology in a systematic and controlled manner and represent simplified and abstracted versions of behavior and physiology, which can be used to develop hypotheses applicable to humans and/or to test hypotheses originating from clinical work [34].

V. TYPES OF ANIMAL MODELS

The following overlapping categories of animal models [29,36–39] have been proposed.

A. Behavioral Similarity Models

These types of models are designed to simulate specific symptoms of a human disorder in animals. The primary intent is to produce a particular set of behaviors that are similar to those shown by humans with a certain illness, rather than to evaluate any specific etiological theory or to study underlying mechanisms or even treatment responsiveness. The validity of these models is judged by how closely the model approximates the human disorder from a phenomenological standpoint [29]. Inducing conditions became secondary.

B. Theory-Driven Models

In these approaches a theory drives the development of specific experimental paradigms. One does not assume the validity of the theory in order to proceed with the research. Rather, the goal is to operationalize the theory one wants to evaluate and study prospectively

the efforts of specific manipulations designed to represent putative causative factors.

C. Mechanistic Models

In these kinds of models, animals are used to study mechanisms. With the increasing array of methods for studying pathophysiology, there has been a preoccupation with the molecular and submolecular basis of altered behavior seen in many animal models. Some would consider that the only useful animal models are those which permit these types of studies.

While mechanistic studies can include evaluation of both neurobiological mechanisms as well as social, behavioral, and developmental mechanisms, one cannot necessarily transpose techniques of mechanism studies cross species, i.e., from humans to rodents to primates or vice versa from rodents to monkeys. The study of mechanisms needs to be specific for a particular species. A serious challenge for animal modeling research is the development and utilization of techniques for mechanism studies in socially behaving animals. Some compromises between invasiveness of neurobiological studies and assessment of social behavior may be necessary [29]. Insistence on cross-species mechanistic similarities is premature given that at present we have no mental disorders in humans uniquely linked with a specific mechanism.

D. Empirical Validity Models

Perhaps the best-known and widest use of animal models involves the use of animal preparations to develop and test potential clinically active drugs. In this context, an ideal animal model is one in which there are no false positives and no false negatives; that is, when a drug works in animals it is predictive of its clinical effects in humans, and when it is inactive in animal models it will not have clinical efficacy in humans. Although there are a number of models with high empirical validity, there is never 100% correspondence between the effects of a drug in an animal model and in a clinical condition. The establishment of an animal model as valid on empirical grounds (or on any other grounds) does not necessarily establish its validity on other parameters.

E. Genetic Models

Genetic models involve studying strains that exhibit spontaneous behaviors that mimic a given illness. Through selective breeding, some investigators have developed animal strains that are especially sensitive on certain tests. This topic is discussed more extensively elsewhere in this chapter.

VI. VALIDATION CRITERIA FOR ANIMAL MODELS

In 1969 McKinney and Bunney [36] made explicit the concept of using animal models for studying human depression and proposed for the first time criteria to consider in developing and evaluating animal models in general. Subsequently, modified or expanded sets of criteria were presented [5].

Willner [39] has described three different concepts of validity:

1. *Predictive validity* primarily concerns the correspondence between drug actions in the animal model and in clinical situations. Manipulations which have certain effects in humans should have similar effects in the animal model for that model to be valid from this standpoint. Using this criterion, there will always be false positives and false negatives. Not all agents that work in an animal model will also work in humans, and not all drugs that work in humans will necessarily work in animal models. There is no animal model that has perfect concordance in this regard. In terms of evaluating animal models according to this criteria it is the pharmacological profiling that is critical rather than the response to just one drug.
2. *Face validity* means that there are phenomenological similarities between the model and the illness being studied. In any one model it is never possible to model all the composite patterns of behaviors shown rather than the presence or absence of any one behavior or symptom.
3. *Construct validity* refers to the theoretical rationale for the model, which in turn relates to the theoretical understanding of the clinical condition and its causation. Unfortunately, too many proposed animal models utilize single proposed etiologies rather than a concept involving multiple risk factors. One of the exciting challenges for future animal models is the evaluation of the relative contributions of various risk factors thought to be important in the human syndrome in question.

Geyer [1] makes the point that, before criteria can be considered, it is important to be explicit about the intended purpose of the model which will determine in part the criteria that should be utilized in evaluating its

validity. He contends that for a model to be of value in it must satisfy only two criteria: reliability and predictive validity. He does not think that construct validity is essential.

VII. SIGNIFICANCE OF ANIMAL MODELS

As Willner has stated [37,39,40], animal models form an important interface between clinical psychiatry and basic research in behavioral neuroscience. In this context they represent major modalities by which developments at the basic level can be brought into a clinical perspective and clinical theories can be evaluated in a controlled manner. This viewpoint contrasts sharply with the position that animal models have no more use since, for example, we can now discover and design drugs that are very specific and can go directly to testing in humans.

The significance of animal models is also their role in the specification and study of focused components of a clinical syndrome. Experimental paradigms in animals permit evaluation of selected aspects of human psychopathology in a systematic and controlled manner. Their obvious advantage is in the ability to precisely control and alter inducing conditions and to permit the collection of prospective data on both a short- and a long-term basis and permit a broader range of mechanistic studies. For example, in relation to depression, prospective studies examining the effects of developmental events on behavior and on neurobiology can be done much more easily in animals. The timing and exact nature of certain alterations in development can be specified, and the short- and long-term consequences studied. That aspect of modeling research is relevant to the question of developmental vulnerability based on early experiences and the mediating mechanisms of vulnerability.

Animal models make possible the dissection of mechanisms in a more direct way than is possible in human clinical research, and they complement ongoing efforts in human protocols, although such procedures need to be suited to both the species and the overall purpose of the experimental paradigm. The research questions have to be clear and specific.

It is easier in animal studies to isolate and evaluate single variables in terms of their main effects and their interaction with each other. For example, the nature of the interactions among genetic, developmental, social, and biological variables can be studied in various combinations in different species. In human clinical research, multiple variables interact simultaneously, and it has been virtually impossible to sort them out in any quantifiable way.

Of course, animal models are most widely utilized in the preclinical evaluation of drugs. A related aspect is their contribution to a better understanding of the mechanism of the action of drugs in altering specific behavior patterns that goes beyond a mere prediction of whether drugs work or not [29].

Studies utilizing animal models can also help to understand the mechanisms of established treatment techniques, i.e., why do some treatment work in certain paradigms whereas others do not? A type of significance which is often not recognized is that animal modeling research has led to the development of improved behavioral, ethologically based rating methods that are now widely used in clinical research settings.

The following quote is focused on affective disorders but, when considering the significance of animal models for psychopathology in general, contains principles applicable to any psychiatric disorder:

> The traditional difficulties in accepting animal models for psychopathology stem from the argument that there is no evidence for what occurs in the brain of the animal that is equivalent to what occurs in the brain of a human. However, if one models any or some core aspects of affective disorder, this model can become an invaluable tool in the analysis of the multitude of causes, genetic, environmental or pharmacological, that can bring about symptoms homologous to those of patients with affective disorders. Animal models can also allow the study of the mechanisms of specific behaviors, their pathophysiology, and can aid to develop and predict therapeutic response to pharmacological agents.
>
> The use of animal models in the research of affective disorders is multifold. Firstly, these models offer experimental systems that may provide insights into the multitude of causes, genetic, environmental or pharmacological, that can bring about symptoms homologous to those of patients with affective disorders. Models also allow study of the development of specific behaviors and their underlying neuroanatomical substrates and neurochemical mechanisms. Finally, animal models can be utilized to develop and predict therapeutic response to pharmacological agents and investigate their putative mechanisms of action [41].

VIII. CHANGING ROLE OF ANIMAL MODELS

A. Neurosciences

Rapid developments in the basic and clinical neurosciences have presented and will continue to present opportunities yet also serious challenges for animal modeling research. On the positive side has been the general recognition of the importance of having experimental animal models if one is going to better understand the pathophysiology of psychiatric illness and move beyond correlative research. Indeed, some contend that the only useful animal models are those that permit molecular mechanistic studies to be done. However, as important as these types of models are, they are not the only useful types of animal models. There is also a role for more integrative models that will facilitate the study of vulnerability factors in a broader context. Conceptualizing animal models narrowly in a deterministic basic neuroscience context has had some unfortunate consequences in terms of the field's development in that attention to the development and study of new biobehavioral models has been diminished along with critical research on already existing models.

"Mechanisms" should not be viewed as synonymous with "molecular." There is far more involved in understanding mechanism of behavior than molecular genetics and molecular biology. Some animal models will lend themselves to molecular biological studies of mechanisms; others will allow other kinds of contributions. Many major discoveries that have significantly impacted clinical psychiatry have come from either behaviorally oriented studies in animals, e.g., the significant enhancement of our understanding of developmental theories and attachment systems, and/or have been based on empirical observations of animal behaviors in relation to drug treatment, e.g., the initial observations by Cade of lithium's calming effects in guinea pigs [42].

A major challenge/opportunity for the development of animal models in the future relates to alterations of circadian rhythms which remain among the most pervasive and consistent findings in several types of mental disorders, especially the mood disorders. A considerable amount of research needs to be done to understand the mechanisms that underlie this connection. One context to begin to understand these mechanisms is at the interface between development/early experience, social stress, and circadian rhythms. This approach could serve as the nexus of a new approach to animal models which incorporates genetic, developmental, and social stress issues. With the identification and characterization of the first mammalian circadian clock gene [43], some exciting cross-species approaches with high relevance to human disorders are going to become possible.

B. Stress Vulnerability

Early theories of the origins of human psychopathology focused on the importance of a variety of early life relationships and events. With the advent of a new era of neurosciences, interest in such developmental events has waned in many quarters. However, research utilizing animal models over the past 25 years has continued to steadily emphasize that these development stressors are important and can have long-term effects on brain and neurobiological development [44–47]. Obviously such events do not operate in a vacuum. The role of genetic vulnerability and how this interacts with developmental events and their consequences is an extremely important and newly emerging area of research in which experimental animal models can play an increasingly important role. Major theories have been proposed that provide an integrated developmental neurobiological perspective of depressive disorders [48–51] and of schizophrenia [52]. In terms of this approach, animal models have already contributed and have great potential for the future [13,53–59].

C. Clinical Disorders

At present, diagnostic criteria for most human clinical disorders involve both a time dimension and signs and symptoms. Since the defining criteria for animal models of psychiatric disorders rely heavily on observed behaviors, a research challenge is to operationalize in animals what in humans are reported as subjective symptoms. Of course, an animal cannot tell one whether it has a certain symptom or not; however, it is possible to measure in animals such things as motor activity, food and water intake, weight, sleep, a range of social activities, changes in self-rewarding activities, and cognitive behaviors. A collection of changes in such behaviors might be postulated to resemble the symptoms or behavioral changes shown by humans diagnosed as having a certain illness, and by working within proper ethological frameworks cross-species research can aid in the understanding of human illness.

Rather than trying to model an aggregate of symptoms, another approach is to focus on the experimental production of a more limited set of behaviors and to

use animal preparations to study these behaviors, e.g., anhedonia, uncontrollability, or changes in social and self-directed behaviors.

What induction techniques to use? There are two broad approaches in utilizing induction procedures. One approach is to use those for which there are data to suggest that they might be important in the etiology of human clinical disorders. An alternative approach is to not worry too much about the cross-species compatibility of inducing conditions, but to use procedures that will produce a set of behaviors in animals that bear some phenomenological similarity to a human illness.

IX. GENETICS

Statements are beginning to appear that the best, or even only, approach to animal models is "genetic," though it is not totally clear what this means. However, with the established importance of genetic vulnerability, experimental paradigms to systematically study genetic variables in animal models are needed as part of an overall approach to the creation and development of animal models. Many techniques are possible. For example, two lines of work, both from the animal models of depression literature, can be summarized as illustrative of this approach. One involves selective breeding and the other study of a specified strain [5].

A. Selective Breeding: Flinders–Sensitive Line (FSL) of Rats

The Flinders line rats [17,60,61] were developed by selective breeding for differences in effects of the anticholinesterase, di-isopropylfluorophosphate (DFP) on temperature, drinking, and body weight. The FSL line rats are more sensitive to DFP as well as cholinergic agonists and have more brain muscarinic receptors in comparison with the Flinders-resistant line (FRL). They were originally proposed as an animal model of depression because of reports that human depressives are also more sensitive to cholinergic agonists. The FSL rats also resemble depressed humans in some other ways: elevated REM sleep, appetite and weight changes, reduced activity and increased anhedonia after exposure to chronic mild stress, and exaggerated immobility in the forced swim test. Imipramine, desipramine, and sertraline have all been shown to reduce immobility in the forced swim test in the Flinders line rats. Lithium, bright lights, and DFP do not. Likewise, amphetamine and scopolamine have no effect in the forced swim paradigm. The calcium channel blockers verapamil and nicardipine were effective in reducing immobility in the forced swim test. Overstreet [62] has also presented data that the FSL rats exhibit altered sensitivity to the locomotor suppressant effects of diazepam; however, anxiolytic effects of diazepam are similar in the FSL. They do not voluntarily drink much alcohol, unlike some depressed individuals. They also do not exhibit any model schizophrenic behavior. They found that swim test immobility cosegregates with serotonergic but not cholinergic sensitivity in cross breeds of Flinders line rats. In conclusion, they present the FSL rat as fulfilling the criteria of face, construct, and predictive validity for an animal model of depression.

B. Specified Strain: Wistar Kyoto (WKY) Rats

Okamoto and Aoki [63] isolated a strain of Wistar rats with spontaneously developed hypertension, the SHR rat. Its normotensive inbred progenitor strain, the WKY rat not only differs from the SHR in respect to resting blood pressure, but also displays smaller stress-induced increases in plasma catecholamines [64], heart rate, and blood pressure [65–67]. In contrast, WKYs show larger endocrine and behavioral responses to stress than SHRs and a heightened susceptibility to stress ulcer. WKY rats [18,68,69] have been proposed as another animal model of depression based on the fact that they (1) exhibit hypoactivity in open field and defensive burying tests (2) readily acquire a learned helplessness task as well as a passive avoidance task; and (3) exhibit more depressive behavior in the Porsolt forced swim test of "behavioral despair" and desipramine reduces the immobility seen in this test. WKY rats also have a heightened susceptibility to stress ulcer and show evidence of heightened emotionality and an exaggerated stress response.

C. Other Genetic Strategies

Another genetic approach would be to utilize targeted mutagenic strategies that rely on transgenic and recombinant DNA-based knockout technologies to create animal models in available biobehavioral tests, thus permitting, within the limitation of these strategies, better understanding of the role of various genes in the control of specific behaviors. A critical research challenge for the future is the question of what specific

strategies should be used to develop such models based on current knowledge of the pathophysiology of various mental disorders.

Other genetic approaches could involve genetic manipulation of candidate genes leading to knockout or transgenic mice, chance findings of altered behavioral phenotypes in other, not a priori designed, mouse mutants, or systematic behavioral screening of mutagenized mice to gain novel animal models [41]. A theoretical advantage of the transgenic or knockout approaches is that a specified behavioral alteration can be assigned to a single gene mutation. However, compensatory mechanisms and genetic background are always at work and sometimes obscure the role of a specific gene in a behavior.

A method that will gain influence over the next years is genome-wide or directed mutagenesis followed by screens for relevant phenotypes. The forced swim test has already been used as a pilot behavioral assay in a random mutagenesis screen [70], but the identification of a series of well-defined and well characterized tests with high predictive validity would dramatically increase the efficacy of such an enterprise.

X. NEW THERAPIES

A. New Methods of Discovering and Developing Pharmacological Therapies

Drug discovery is a multidisciplinary effort requiring chemical, structural, and biological approaches. This last includes animal models.

Historically, one of the major uses of animal models has been for the preclinical screening of proposed pharmacological treatment agents. In this context, a variety of experimental animal models have been developed which have reasonable empirical validity. Unfortunately, the presence of false negatives and false positives has led some to sharply criticize animal models and even refuse to use them in drug discovery and development.

A related position is that animal models, in the context of drug discovery and development, are irrelevant given newer molecular techniques for discovering and developing drugs. Newer therapies can be discovered based on hypothesized molecular mechanisms of illness and then moved directly to clinical trials. However, one of the major problems with this approach is that not enough is yet known about the specific pathophysiology of psychiatric illnesses to let that alone drive the therapeutic discovery and development process. Some in vivo testing in animals remains critical to complement drug discovery based on novel mechanisms. Also, since psychiatric disorders are still largely defined by behaviors, it does not intuitively make sense to bypass behavior in the drug discovery and development process [5].

XI. ANIMAL RIGHTS ISSUES

This is one of the foremost challenges with regard to animal modeling research [71,72]. The use of animals for biomedical research in general, and especially for neuropsychiatric disorders, continues under serious threat [73–80]. Detailed discussion of the various groups and strategies is beyond the scope of this chapter; however, the issue is not animal welfare organizations who, in so many invaluable ways, help look after the welfare of needy animals and deserve our enthusiastic support. Likewise, the problem is not the thoughtful groups that share our genuine concern about the welfare of all animals and work to establish reasonable regulations. The problem is with those organizations that are dedicated to stopping animal research at all costs, including violence to researchers and to physical property, and who advocate senseless bureaucracy to discourage researchers from pursuing animal research. We all support the humane treatment of all animals in research and careful and diligent review of all research by independent groups—as is done with human clinical research. However, with the escalating tempo of violence and intimidation in this area that has occurred over the last 10–20 years, some government agencies have hesitated to move ahead with programmatic initiatives in the animal modeling research area, and some universities have been, at best, ambivalent in backing faculty doing animal research. The field has lost productive people as a result, and new, junior people have sometimes hesitated to enter the field. This is a major challenge for the field in the future.

XII. SUMMARY

There are a variety of animal models available for many psychiatric disorders. Just as in any other field of medicine, none are perfect. Indeed, if they were, they would be replicas rather than models. Continuing efforts need to be made to further understand and utilize the models that are available as well as to develop new ones. However, major challenges for the

future will also include dealing with the conceptual and philosophical issues that surround animal modeling research in psychiatry. Many of these have been summarized in this chapter.

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2

Methodological Advances in Psychiatric Genetics

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I. RATIONALE FOR GENETIC RESEARCH IN PSYCHIATRY

Genes play a major role in determining and controlling every phenomenon in life. The inherited potential of the new embryo is encoded in the genes transmitted to him by both his parents. The activation or deactivation of certain genes at certain times governs the differentiation of embryonic stem cells into different tissues and systems. This influence goes on after birth and throughought the life span when the production of enzymes and structural proteins is under genetic control. In this way genes control the activity of cells, tissues, and body systems, produce disorders, and determine programmed cell death. Genes also mediate the influence of the environment. Nutrition, toxins, infectious agents, and psychosocial stresses can all affect the organism by activating or deactivating certain genes. Also, genes may affect the environment that a subject is exposed to. For example, it was found that monkeys with an inborn tendency to have low levels of serotonin metabolite in the CSF were more likely to be subject to violent death in a younger age [1].

When we try to better understand the etiology and pathogenesis of complex phenomena such as behavior, personality traits, and psychiatric disorders, it seems almost impossible to disentangle the numerous factors involved in their development. The situation is very different from research on metabolic disorders, where a biochemical imbalance is obvious and allows rapid characterization of the enzymatic defect and its etiology. In the case of schizophrenia numerous changes probably occur, from the hypothesized maldevelopment of the embryonic central nervous system during the first trimester of pregnancy until the onset of disease at the age of 15–20 years. That psychotic symptoms improve after the administration of dopamine D2 receptor antagonists tells us very little about the beginning of the process two decades before, nor can it help us define the contribution of inherited and environmental factors to the development of the illness.

This complexity of mental phenomena makes genetic research crucial to their understanding. Genes have been implicated in the etiology of almost every psychiatric disorder. The etiological role of environmental and psychosocial factors is also well recognized and, as noted above, may be substantially mediated through genes. Unlike environment, which is constantly changing and always difficult to characterize, genes remain unchanged for the most part from conception until death. Although the level of activation of genes does change throughout life, the DNA sequence remains practically constant. Thus we can find in the adult person with schizophrenia the same inherited predisposing genes that started the process of the disorder in fetal life. Identifying these genes would allow

us to understand the unfolding of this process and characterize the relative contribution of environmental factors. Ultimately, this would lead to improvement not only of diagnosis and treatment, but also of prevention. The strategy of starting research on the etiology of disorders by first finding the contributing genes, and then revealing the pathogenesis, is opposite in direction from classical research in medicine and therefore is called ''reverse genetics.'' This methodology will be described in the following sections and is summarized in Table 1.

II. DEFINITION OF PHENOTYPES

Before a search for a disease-causing gene begins, one should correctly define the phenotype—who has the disease and who does not. It is widely aknowledged that psychiatric diagnosis is limited to subjective measures. The identification of genes that cause psychiatric disorders should improve our diagnostic capabilities imensely. The circular problem is that in order to find these genes we should make the correct diagnosis. Broadening the diagnosis to include as many individuals as possible with similar symptoms is doomed to hamper our effort to find the gene. It was shown, for example, that the transmission of schizophrenia is independent of the transmission of bipolar disorder [2]. But even categories such as DSM-IV-defined schizophrenia might be too broad. It is concievable that what we define today as one disorder comprised dozens of different diseases with different etiologies and clinical pictures. Narrowing down the diagnosis is an important step in ensuring that a homogeneous sample with the same genetic etiology is studied. This process should be based not only on theoretical hypotheses but on empirical data. Thus, patients can be subdivided according to clusters of specific symptoms (such as negative or positive ones), neuropsychological tests or imaging studies (e.g., enlarged or nonenlarged ventricles). On the other hand, too much narrowing could lead us to false-negative results and to missing a gene that is expressed differently in different individuals. For example, the genetic liability to develop schizophrenia can also lead to a spectrum of related disorders such as schizotypal and schizoid personality disorders [3].

III. ESTABLISHMENT OF GENETIC ETIOLOGY IN PSYCHIATRIC DISORDERS

A. Family Studies

The first step in genetic research on any disease is to establish its heritability. The most obvious way of doing so is by studying the recurrence rate of the disorder in relatives of affected individuals. This rate should not be measured simply against the rate in the general population. Rather, the comparison should come from the study of relatives of healthy controls, who should be diagnosed using the same instruments. Raters should be blind to the proband's diagnosis. Family studies have revealed increased risk in relatives of probands with schizophrenia [2,4], bipolar disorder [2], major depressive disorder [5], obsessive-compulsive disorder [6], autism [7], attention deficit hyperactivity disorder [8], and anorexia nervosa [9], among others. Increased risk for relatives would suggest that the disorder is familial. This is not equivalent to its being genetic since family members share more than genes. Environmental factors such as nutrition, infections, and psychosocial stressors are also common to family members.

Table 1 Methods of Identifying Genes for Medical Disorders

Establishment of Heritability	Localization of Genes
Family studies	Parametric Linkage Analysis
Adoption studies	Nonparametric methods
Twin studies	Association studies: case control and family based
Segregation analysis	Haplotype relative risk (HRR)
	Transmission disequilibrium test (TDT)
	Allele sharing methods (sibpairs and pedigrees)
	Linkage disequilibrium in genetic isolates
	Quantitative trait loci (QTL)

B. Adoption Studies

Adoption studies are designed to try to disentangle inherited from environmental etiological factors. The rate of the studied disorder is compared between biological and adoptive parents of individuals with a certain disorder. Alternatively, the risk of the disorder can be compared between offspring of affected individuals who were given for adoption and offspring of healthy individuals who were also given for adoption. A third and obviously rare paradigm would be the comparison of offspring of healthy parents who were either adopted by affected individuals or by healthy ones. The last variation is the study of offspring of affected parents who were either reared by them or adopted or reared outside their families (as in foster homes). These methods have mainly been used for the study of schizophrenia [3,10], bipolar disorder, and major depressive disorder [11,12], and have shown the major role of genetics in their etiology.

C. Twin Studies

Twin studies are the gold standard of research on the genetic etiology of disease. The recurrence risk or concordance of a disorder is compared between monozygotic (MZ) and dizygotic (DZ) twins. MZ twins share 100% of their genes while DZ twins share on average 50% of their genes, as do any other pair of siblings. Researchers assume that MZ and DZ twins are similar in the degree to which they share environmental influences. While it is probably true that twins are exposed to the same kind of environment more than nontwin siblings, it is obvious that MZ twins experience a more unique and shared environment than DZ twins.

The only way to overcome this limitation is by comparing concordance rates of MZ twins who were reared together or apart. As this is a rare phenomenon, it is quite impractical for genetic research. Nevertheless, twin studies are considered the best estimate of the heritability of the disorder, which is calculated from the difference in concordance between MZ and DZ twins. Thus, autism is considered to be highly heritable with an MZ concordance rate of 92% compared to 10% for DZ twins [13]. Bipolar disorder has also a high heritabilty rate with MZ concordance of 67% against 20% for DZ twins [14]. In schizophrenia the rates are also suggestive of a strong genetic component in etiology: 48% concordance in MZ twins and 4% in DZ twins [15]. Concordance rates also teach us about the importance of environment in the etiology of disorders. The 48% concordance rate for schizophrenia between MZ twins is a striking example. While a strong genetic influence is suggested, it also means that a person with the genetic predisposition to develop the disorder has $>50\%$ chance of avoiding it, perhaps by avoiding a noxious environment or by experiencing some as yet unknown, protective factors. The study of discordant sibs or preferably discordant MZ twins can thus teach us on the role of environment. For example, it was shown that MZ twins with Tourette syndrome (TS), a neuropsychiatric disorder characterized by motor and vocal tics, varied in the severity of symptoms. The twins with a more severe clinical course had a lower birth weight [16].

D. Segregation Analysis

After establishing the role of genetic factors in the etiology of a disorder, the next step is to try to define its mode of inheritance. This is done by studying large pedigrees with multiple affected individuals. The observed inheritance is compared with expected inheritance under various genetic models. The goodness of fit is calculated and certain solutions are rejected. Those that are not rejected are considered consistent with the data, which supports this solution as a possible mode of inheritance (although it does not prove it to be the right one). Using this method, most psychiatric disorders show a complex mode of inheritance. None of them is consistent with simple Mendelian inheritance (i.e., autosomal dominant or recessive, X-linked). Even models of oligogenic or polygenic inheritance (few or many genes with small contribution of each of them) must take into account the role of environment to fit the data. Thus, the model consistent with the inheritance of psychiatric disorders is usually termed multifactorial [17]. The impact of this on the choice of methods for genetic analysis is discussed below.

IV. LOCALIZATION OF GENES THAT PREDISPOSE TO PSYCHIATRIC DISORDERS

A. Parametric Methods

Until a decade ago the most widely used method employed to detect genes for inherited disorders was parametric linkage analysis. In this method large pedigrees with multiple affected members are studied. In each pedigree the inheritance of the studied disorder is compared with the inheritance of DNA markers with a known location on the human genome. The marker

can be chosen because of an a priori idea regarding the genetic location of the disease gene. This idea might stem from the study of affected individuals with chromosomal aberrations. This was the case in Douchene muscular dystrophy (DMD). The gene for this X-linked disorder was localized after the study of two females who had the disorder were found to have a deletion and a translocation in a certain region on chromosome X [18]. More frequently there is no idea about the putative location of the disease gene. In this case DNA markers spanning the whole genome are used in what is called a genome scan. These markers are usually DNA sequences with no genetic function known to us, but with slight variations from person to person. These variations, or polymorphisms, serve to mark a specific location on the human genome. Depending on the number of markers (usually in the order of hundreds), some gaps are left unchecked. Nevertheless, as nearby genes and markers are usually transmitted together from parent to offspring, we can compare the inheritance of the marker and of the disease in a certain pedigree. If the studied disease is inherited together with a specific marker, we have a clue about the location of the disease gene.

Take for example the hypothetical pedigree in Figure 1. From looking at the pedigree it seems that the inheritance of the disorder is indeed linked to the inheritance of the studied marker. The affected grandfather (#400) has transmitted the disease coupled with allele 1 of the marker to some of his offspring. The

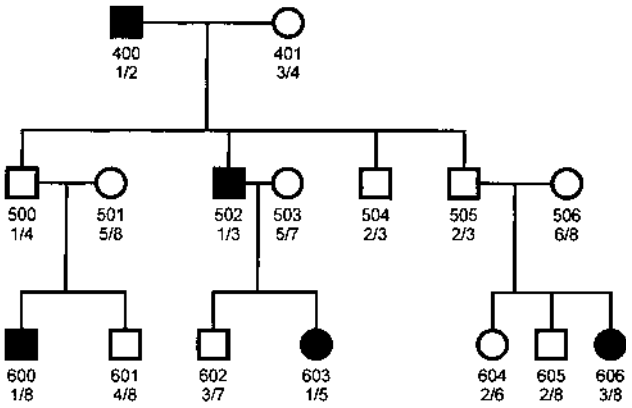

Figure 1 A hypothetical pedigree for linkage analysis. Affected individuals are marked by a dark symbol. Individuals are given identifying numbers from 400 to 606. Genotypes for a certain marker are shown for each individual by the two alleles found in this individual.

possibility that true linkage exists between the two phenomena is compared with the possibility of observing this by chance. The ratio between these two probabilities is calculated. A ratio of 1000 in favor of linkage is traditionally considered significant. For practical reasons the ratio logarithm is used and called the LOD (logarithm of the odds) score. The LOD scores of different pedigrees can be summed together. Tight linkage (LOD score of >3) implies that the disease gene is located close to the studied marker. LOD score of -2 excludes the region as the possible location for the disease gene. When a genome scan is carried out, multiple testing for hundreds of markers is being done and thus a higher level of significance is needed. A LOD score of 3.3 was shown to correlate to a P value of .05 in this situation and is thus considered significant evidence for linkage in a genome scan [19]. LOD scores of 1.9, the magnitude of most positive findings in psychiatry, are considered only suggestive of linkage.

Recombination, the exchange of DNA between a pair of chromosomes during meiosis, can decrease the evidence for linkage by separating the disease gene from the linked allele. Thus, in our hypothetical pedigree the disease gene can be transmitted by individual #400 to one of his offspring with allele 2 instead of allele 1. This might decrease the evidence for linkage in the pedigree. To overcome the problem, the recombination rate is allowed for in the calculation of the LOD score in relation to the estimated distance between the disease gene and the marker. The recombination rate (or fraction) can vary from 0% if they are in exactly the same location to 50% (or 0.5) if they are on different chromosomes.

As stated above, parametric linkage analysis was the main method of genetic analysis employed until recently. It led to the discovery of genes causing disorders with simple Mendelian inheritance such cystic fibrosis [20] and Huntington's disease [21]. In disorders with a more complex inheritance, such as diabetes, hypertension, and psychiatric disorders, it has not proved to be as useful. The main limitation of this method is that in order to correctly calculate the probability of linkage, certain parameters regarding the inheritance of the disease have to be taken into account. First of all, the mode of inheritance has to be specified. As noted before, in psychiatric disorders, mode of inheritance is probably polygenic with a considerable environmental contribution.

For many years the genes for psychiatric disorders were sought under the incorrect assumption of simple Mendelian inheritance, which yielded mainly negative results or some unreplicable positive results. For exam-

ple, researchers who studied Tourette syndrome, were very optimistic at first regarding the chances of finding the gene causing the disorder. It seemed that the inheritance of the disorder was autosomal dominant. It took many years and a great deal of effort with no significant results of parametric linkage analysis to realize that even in TS the inheritance is probably more complex [22,23]. As the exact number of genes that act together to cause psychiatric disorders and the relative role of environment are not known, it is very hard to define the right model for linkage calculations. Unfortunately, the only way to overcome this problem is by finding these genes and isolating their etiological influence from that of environment.

Mode of inheritance is only one of the parameters that linkage analysis is dependent upon. Other parameters are also not known for psychiatric disorders and have to be guessed. Gene frequency, for example, is estimated from disease frequency and the number of implicated genes. The rate of genetic heterogeneity is also estimated. This is the proportion of pedigrees in the studied sample where the disease is caused by different genes. Penetrance has to be taken into account as well. This is the probability that a certain person who carries the disease gene will actually express the disorder. Correct definition of the phenotype is a serious problem that limits the use of parametric linkage in psychiatric genetics, and has been already discussed. But even if we had an accurate diagnostic measure, we would probably still encounter people who carry the gene but for some reason, such as protective factors, do not express the disorder. This might be the case of the person in Figure 1 (#500) who transmitted the disease gene with the linked allele to his affected son from his affected father. Thus, with no correction for penetrance, true linkage can be missed.

Another related parameter that has to be specified in linkage analysis is the rate of phenocopies. These are affected members in the pedigree who acquired their disease because of another, nongenetic factor. This might be the explanation for the occurrence of the disease in individual #606 in the hypothetical pedigree in Figure 1. She has the disease but not the 1 allele. Another possible explanation is that this woman inherited the disease gene or another gene causing the disorder from her mother who is unrelated genetically to the affected grandfather. This phenomenon of disease genes coming from different founders of the pedigree is called bilineality. It is common in pedigrees with psychiatric disorders where assortative mating (between two affected individuals, or between relatives of affected individuals) is common. Bilineality is another parameter that has to be considered to calculate linkage. Usually, researchers attempt to exclude bilineal families from their samples, but absence of bilineality is very difficult to establish with certainty.

Thus, most of the parameters needed for the calculation of linkage are not known for psychiatric disorders and will be known only after the genes for the disorders have been found. When running parametric linkage analyses, many estimates and guesses are made. For each analysis, a specific model composed of the estimated parameters is being used. The many parameters and the numerous different options for each of them make endless numbers of combinations. Examining all of them is impractical and carries the risk of obtaining false-positive results because of multiple testing. Examining only a limited number of models, as is usually done in linkage analysis, is unlikely to include the correct one. This is probably the reason why parametric linkage analysis has been unable as yet to identify a gene for any of the major psychiatric disorders.

Notwithstanding the limitations of parametric linkage analysis in the study of psychiatric disorders, there might be rare instances where its application can be fruitful. Some rare neuropsychiatric disorders such as Rett's disorder, a severe autisticlike X-linked disease, have a more simple Mendelian inheritance. The gene for Rett's disorder was cloned after linkage analysis had been localized to a certain location on chromosome X [24]. The identification of such disease genes, even if rare, could shed light on the pathogenesis of other more common disorders.

B. Nonparametric Methods

Unlike parametric linkage analysis, nonparametric methods are not dependent upon the specification of parameters regarding inheritance. Thus, their use is more suitable for the study of disorders with complex inheritance. Nonparametric methods have been used extensively in the past decade and have shed light on the genetics of disorders such as diabetes, hypertension, Alzheimer's disease, and psychiatric disorders.

1. Association Studies

Case control association studies overcome the problems encountered in genetic analyses of complex disorders by studying a sample of unrelated affected individuals. In this group the frequency of alleles of certain genes is determined and compared to the frequency of the same alleles in a control group of un-

affected individuals. Because the affected individuals are not related, increased frequency of a certain allele in them does not usually imply linkage with the disease gene. Rather, it points to a direct role of the studied allele in the etiology of the disorder. Because of that, different alleles or DNA polymorphisms of genes must be studied instead of merely studying DNA markers. This means that the DNA variations that can be used in association studies must occur in the regions of the genome that code for proteins. The variations can occur in an exon, which is the coding region of the gene, meaning that sequence encodes the sequence of amino acids in the product protein. In other cases the polymorphism occurs in a noncoding region of the gene, or intron. As noted above, if a polymorphism occurs in a noncoding region between genes, it is not useful for association studies, as linkage to a nearby gene cannot be studied in a group of unrelated subjects.

Polymorphism can also occur in a regulatory region of the gene, which is a sequence of DNA where certain molecules bind and affect the rate of transcription. A functional DNA polymorphism occurs in a coding region of a gene and affects the function of the product protein. Functional polymorphisms, or those occurring in the regulatory region of the gene, are usually preferred in association studies, as genes with a suspected role in etiology of disorders are investigated in this paradigm.

It was thought until recently that association studies were not suitable for a genome scan because they cannot detect linkage. Now, with the completion of the Human Genome Project, almost the entire DNA sequence of the human genome is known. Thus, theoretically, association can be studied in polymorphism in each and every gene. Practical, technical, and computation limitations make this option not feasible yet.

Many association studies of psychiatric disorders have been performed using candidate genes. These are genes for proteins with a hypothetical function in the pathogenesis of the disorder. In psychiatric genetics these candidate genes are usually genes involved in the production, metabolism, and signal transduction of neurotransmitters. For example, a repeat polymorphism in the gene for the dopamine receptor D4 was found to be associated with ADHD [25] and with the novelty-seeking personality trait [26], and a certain allele of the serotonin transporter gene was associated with anxious personality [27]. These associations were significant but of small magnitude. They explained only a small part of the variance of the studied traits. This means that these genes might have a small contribution to the etiology of the studied phenomena. Combinations of alleles for two different genes were studied and shown to be associated with tardive dyskinesia, for example [28]. Looking at larger numbers of genes simultaneously would yield a greater number of combinations that might be too numerous to study without enormous samples.

The relatively small role of each gene found to be associated with disorders is only one limitation of this study design. As the Human Genome Project has revealed, there are ~30,000 genes (many fewer than once thought, but still an enormous number). A third of these genes are estimated to be expressed in the brain. Most of them are still not known. Moreover, our understanding of the pathogenesis of psychiatric disorders is limited to current processes in the brain of the affected individual. These may be very different from the genetic vulnerability that started the disease many years before. A genomewide association scan might overcome these obstacles but, as stated above, is not feasible yet.

Notwithstanding the above-mentioned difficulties of association studies, their main limitation is the need to find a perfectly matched control group. Controls should resemble affected individuals in every measure that might be related to the studied genes, apart from disease status. The most problematic confounding factor in this regard is ethnicity. Variations of allelic distribution are highly dependent on ethnicity. Significant variations of allelic distribution are found even among ethnic subgroups of a relatively homogeneous population such as Jews [29]. The choice of an ethnically unmatched control group might be the reason for the failure to replicate some of the positive associations reported between psychiatric disorders and certain alleles. Indeed, some of these nonreplications were in studies in which the patient and control group came from a homogeneous population [30]. It is hard to say whether the positive results or the nonreplications were spurious. To overcome this problem researchers are turning more and more to methods that employ unaffected family members as controls. Two widely used family based methods are haplotype relative risk (HRR) and the transmission disequilibrium test (TDT).

2. *Haplotype Relative Risk (HRR)*

In HRR allele frequencies are studied in a group of affected individuals. The comparison group is made up of hypothetical sibs of these individuals. These made up sibs are presumably healthy and have inher-

ited from their parents the alleles that were not transmitted to the affected sib (Fig. 2). To be able to make up the control group, both parents of the affected individual must be studied for the same alleles. Thus, HRR requires the ascertainment of "trios" of affected individual and both parents, alive and willing to participate in the study. This makes the research design more complicated. On the other hand, the hypothetical control group in this design is perfectly matched for ethnicity to the patient group.

3. *Transmission Disequilibrium Test (TDT)*

In TDT, as in HRR, trios are needed for the analysis. In this research design, affected individuals with a parent heterozygous for the allele of interest are studied. In each such case it is determined whether the studied allele was transmitted to the affected offspring or not (Fig. 2). A transmission rate that is significantly higher than the random rate of 50% is considered as evidence for a role of the allele in the etiology of the disorder. The inheritance of multiple genes or haplotypes can be investigated as TDT is studied in families.

Although addressing the issue of ethnicity, both HRR and TDT are limited, as association studies in populations, to the study of candidate genes. Nevertheless, both methods are useful for the attempts to further establish findings from population-based association studies. For example, HRR was used to both replicate and (in other populations) not replicate the association of DRD4 with ADHD [31,32].

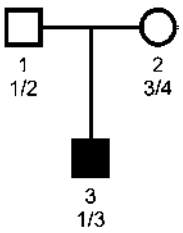

Figure 2 Family based association studies using two different methods. The first method is haplotype relative risk (HRR). Two parents and one offspring (a trio) are studied. The nontransmitted alleles (2 and 4 in this pedigree) comprise the control group of hypothetical healthy sibs made up from many trios. Allele frequencies are compared between groups. The second method is transmission disequilibrium test (TDT). Trios are studied in which one of the parents is heterozygous for a certain allele of the studied marker (1, for example). Transmission of the allele is counted for each trio. The rate of transmission is compared with random rate of transmission, which is 50%.

C. Allele Sharing Methods

1. *General Principles*

In allele sharing methods of genetic analysis, the degree of sharing of alleles of certain genes or markers is examined in related individuals with the investigated disorder. This can be done either in affected sibs, which is called sibpair analysis, or in affected individuals with a more distant relationship (grandfather and grandson, cousins, etc.). Sharing of alleles can be incidental or can be secondary to inheritance of the same allele from a common ancestor (parent, grandparent, etc.). When the reason for the sharing is not known, it is called "identity by state." When it can be shown that both individuals inherited the same allele from a common source, we use the term "identity by descent." Identity by descent allele sharing is a more powerful tool in genetic analysis. If related individuals with the disorder share the same alleles, identical by descent, more often than expected by chance, an association between the allele and the disease is implied. Alternatively, as the individuals are related, increased sharing can stem from linkage of the marker to the disease gene. Thus a genome scan can be performed with these methods, as well as the study of candidate genes.

When allele sharing is studied, no assumption has to be made about mode of inheritance, genetic heterogeneity, gene frequency, penetrance, rate of phenocopies, and so on. This nonparametric method is model free. This is its great advantage over parametric linkage analysis. On the other hand, parametric linkage under the correct model is much more powerful in the detection of linkage. In order to compare their power to that of parametric linkage, allele sharing methods need to use very large samples of the order of hundreds of sibpairs or dozens of multiplex pedigrees. These are hard to ascertain in one population. Researchers usually combine samples from different populations, which increases the risk of genetic heterogeneity and decreases the chance of finding significant linkage. For this reason allele sharing methods are used in combination with parametric linkage analysis, which means that in the same pedigrees both parametric and nonparametric methods are used. For example, the addition of sibpair analysis to parametric linkage analysis was helpful in supporting suggestive linkage to a locus on chromosome 22q in schizophrenia [33]. Significant linkage between two forms of dyslexia and markers on chromosome 6p and 15p were found using parametric linkage for one form and allele sharing methods for the other [34].

2. Sibpair Analysis

Take for example the sibpair in Figure 3. Affected sibs can either inherit the same two alleles from their parents, one shared allele and one nonshared allele, or two different alleles. If there is no relation between affection status and the studied allele, we expect the probabilities for each of the three cases to be 0.25, 0.5, 0.25, respectively.

The actual allele sharing is studied in many sibpairs, and the observed frequency of sharing of one, two, or zero alleles is compared with the theoretical random frequencies. Any deviance from random distribution is considered evidence for increased allele sharing.

3. Allele Sharing in Pedigrees

In this paradigm allele sharing is compared between affected pedigree members with any degree of relationship apart from parent and offspring (as the allele sharing will always be 0.50 in this case). Observed sharing is compared with expected sharing under no association or linkage. As for parametric linkage analysis, large pedigrees with many affected members are ascertained. But unlike traditional linkage analysis, only allele sharing between affected individuals is studied. Take Figure 1 for example. In allele sharing analysis, only the affected individuals (#400, #502, #600, #603, and #606) will be studied, and allele sharing will be examined in every possible combination of two out of these individuals (apart from parent and child). The other unaffected pedigree members do not contribute to the calculation, apart from verifying that identity between individuals is indeed by descent and not by chance. Thus it is clear that this paradigm is model free, as it is not dependent on the parameters required by parametric linkage. The most widely used software that employs this paradigm is called Genehunter [35], which can also calculate parametric linkage in the same pedigree.

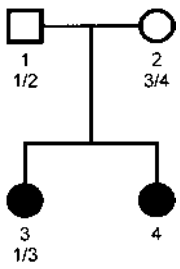

Figure 3 Sibpair analysis: two affected sibs and their parents are studied. In this example one daughter received alleles 1 and 3 from her parents. Her sister can either receive the same two alleles, only one of them (1/4 or 2/3), or the two that were not transmitted to her sister (2/4). The probabilties for each case are equal, and thus for sharing the same two alleles are 0.25, only one allele 0.5, and no sharing 0.25. Actual sharing in many sibpairs is compared to these random probabilities. Significant deviance implies association or linkage.

D. Linkage Disequilibrium in Isolated Populations

One of the main limitations of both parametric and nonparametric methods of genetic analysis is the problem of genetic heterogeneity. Researchers use large samples to increase power, and thus run the risk of mixing subpopulations with different genetic etiologies. Studying small populations that are genetically isolated can overcome this obstacle. In such a population, affected individuals are more likely to represent a homogeneous sample, in terms of etiology. It is plausible that most of the affected individuals in a genetic isolate, who have a certain disorder, carry the same disease-causing mutation, which they inherited from a common ancestor. If the mutation process is relatively new, or more possibly if the genetic isolate is relatively young (and the mutation was introduced to it relatively late), we expect that affected individuals share more than the mutation itself. Large regions of DNA on both sides of the mutation should be identical in these individuals, as the short time that elapsed since the isolate was founded did not allow recombination to change them considerably. Thus, these individuals share not merely alleles but large haplotypes identically by descent. The aggregation of certain alleles into haplotypes that are more frequent than what is expected by chance is called "linkage disequilibrium," and is evidence for the presence of a shared mutation in this region.

The main advantage of this paradigm is that only a few affected individuals (as few three or four) need to be examined. These people do not have to be related (apart from being part of the same isolate), and their relatives are not needed for the study. The disadvantages are that these genetic isolates are not easy to find, and are even more difficult to study. Also, genes responsible for psychiatric disorders in these unique populations might be very well specific to them only. Nevertheless, finding one gene for one psychiatric disorder in one population has not yet been achieved by any other method. Linkage disequilibrium in genetic isolates has been used to locate genes for rare medical disorders with simple Mendelian inheritance [36], and

also for a common disorder with a more complex inheritance such as Hirschprung's disease [37]. Lately, it is being applied to the study of psychiatric disorders as well.

E. Quantitative Trait Loci (QTL)

As implied by its name, QTL is suited for the study of quantitative traits, such as height and weight. It allows the study of many genes, each with a small contribution, to the expression of one continuous variable. QTL is easily applied in laboratory animals, where pure strains can be inbred and the change in the studied trait can be measured under different genetic conditions. Obviously this cannot be done with human beings. Rather, sibs or unrelated subjects with extremely different values of the studied trait are studied and their genotypes compared. It is also debatable whether most psychiatric disorders can be perceived as quantitative traits. Medical psychiatry assumes in most cases a more categorical approach to psychiatric disorders. On the other hand QTL might prove beneficial to the study of personality traits and intelligence [38].

V. ADVANCES IN THE LABORATORY

The significant expansion of DNA marker maps enables the performance of better genome scans. More and more polymorphisms of the human DNA are known. These include single nucleotide polymorphisms (SNP) and certain sequences (from two or three to several dozen nucletodies) that vary in the number in which they are repeated in different individuals. This improved map increases the chances of finding association or linkage with a studied disorder. Data are shared through the Internet and are freely available to any researcher. Technology is improving from day to day. Methods that identify different DNA sequences are much easier to perform and are less time-consuming.

The recent completion of the Human Genome Project opens new and endless horizons for the study of psychiatric genetics. One aspect is the further improvement of marker maps. Moreover, in a short while we should be able (with sufficient computation power) to study association of disorders with each and every of the human genes. Thus we will be able to desert the study of candidate genes that were chosen merely because of our limited knowledge. In this way association studies (both case control and family-based paradigms) will be used for the purpose of hunting genes in genome scans.

When the location of a gene for a psychiatric disorder is eventually found, the new map of the human genome will enable its rapid identification and cloning. The mutations that cause the disorders will be characterized shortly thereafter, and shorten the way to the study of pathogenesis and the application to diagnosis, prevention, and treatment.

VI. SUMMARY AND FUTURE DIRECTIONS

Finding a significant linkage between a psychiatric disorder and a DNA marker, which is replicated consistently, is an objective that has not yet been accomplished. The further objectives of mapping actual disease genes, cloning them, and determining their protein structure and function, are more distant.

The inherent obstacles have been described in this chapter, as well as newer methods of genetic analysis that aim to overcome them. Advances in molecular technology constantly improve our technical tools. Eventually one gene for one psychiatric disorder in (at least) one population will be found. And then? The journey just begins.

The gene for Huntington's disease (HD) was discovered a decade ago [21]. No dramatic change has occurred in the treatment and prognosis of HD patients since then, even though diagnosis is now possible before the onset of the disorder. This has raised painful ethical questions. Is it justified to test young healthy individuals for a dreadful, incurable disease? When we finally find genes for schizophrenia won't we be in the same position? We will have to deal with difficult ethical questions regarding prenatal diagnosis of susceptibility to a disorder with a variable clinical course that has its onset 15–20 years later.

We will also be at the very beginning of the long road which will need to be traversed in order to understand how genes start the process that eventually culminates in psychiatric disorder. Apart from the complexities inherent to the field of psychiatric genetics, there are additional ones. These are related to the fact that interposed between the gene and the protein for which it codes are variations in transcription, translation, and posttranslational modifications that prevent us from being able to assume a simple relationship between gene and disease. Also, epigenetic factors modify expression of genes in ways that can be time specific and tissue specific. Thus, even when a gene for a psychiatric disease is eventually found, a

long route will have to be traversed before we understand how it affects the disease process.

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3

New Developments in the Regulation of Monoaminergic Neurotransmission*

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I. INTRODUCTION

The process of synaptic transmission is the key target for all psychoactive drugs. Transmission may be influenced by drugs affecting the synthesis, storage, release, inactivation, and postsynaptic effects of transmitter substances. Further, drugs effective in major psychiatric illnesses such as depression and schizophrenia have prominent effects on transmission mediated by biogenic amines such as dopamine (DA), norepinephrine (NE), and 5-hydroxytryptamine (5HT; serotonin). The past decade has seen marked advances in our understanding of key features of the transmission process mediated by these amines. Of particular importance is the emerging concept that transmission mediated by these substances appears, at least in part, to occur through diffusion-mediated signaling, termed extrasynaptic or volume transmission (VT). Also, it is now recognized that the inactivation process of reuptake, mediated by specific transporters located in the plasma membrane, plays the key role in regulating the concentration of these amines in the extracellular fluid (ECF). Furthermore, these protein transporters are not merely constitutive membrane components but undergo a variety of regulatory processes. Finally, in the past decade it has become more accepted, even if still not completely understood, that effects of released amines can be influenced by other peptide transmitters colocalized in the same neurons. Our emerging concepts of the functioning of transporters and the processes of cotransmission and VT have not been well integrated into current views of psychoactive drug action. Yet it is likely that they influence profoundly the effects produced by such drugs. Because of this, it is appropriate to view such processes from the perspective of their potential neuropsychopharmacologic impact.

II. HARD-WIRED VS. PARACRINE OR VOLUME TRANSMISSION

The most widely accepted model for synaptic transmission, including that which occurs in brain, was devel-

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oped from studies involving cholinergic transmission through nicotinic receptors, particularly at the neuromuscular junction. This model was derived in part from the morphological characteristics of such synapses, involving a presynaptic knob with specialized features, a cleft of $\sim$ 40–60 nm, and the postsynaptic membrane containing both receptors and invaginations. Such specialized features within the synapse pose barriers to transmitter diffusion and help to ensure that the transmitter acts only within the strict confines of such conventional synapses. Further contributing to acetylcholine (ACh) having only synaptic effects is the presence of its degradative enzyme acetylcholinesterase within the synapse. This type of transmitter process has been termed "hard-wired."

However, in the mid-1970s, anatomic studies on brain tissue generated data that were interpreted as favoring a different model of synaptic transmission. This model has been referred to as extrasynaptic communication or paracrine transmission (which, historically, relates to a hormone affecting the function of cells at a distance from its site of release) or volume transmission (VT). The essence of such transmission is the passage of chemical messages along multiple, largely unpredictable channels such that transmitters may pervade the extracellular space to act at distant receptors outside the strict confines of conventional synapses. Although there are attractive features of this concept, it has been elusive and difficult to prove. It is outside the scope of this chapter to review this subject in detail. The interested reader is referred to comprehensive reviews of this topic in a recent volume [1]. Since the mid-1970s, anatomic, physiologic, and pharmacologic data have been generated that are consistent with VT, although not proving it. If such transmission does occur in the brain, it could have profound neuropsychopharmacologic implications.

The original observation that there may be nontraditional types of transmission in brain was that of Descarries et al. [2]. These investigators, using ^{3}H-5HT autoradiography in the neocortex of rats, claimed that serotonergic terminals were rarely engaged in morphologically differentiated synapses and speculated about "nonsynaptic" release of 5HT in this brain area. Subsequently, Beaudet and Descarries [3] suggested that 5HT acted on a large number of cortical cells rather than just a restricted number of postsynaptic targets. Their notion was of a predominantly nonjunctional serotonergic innervation of the cortex having paracrine-like properties. Although this work has been criticized [4] and others have found much higher percentages of typical synaptic specializations for 5HT [5,6], there does seem to be a body of data showing a reasonable percentage of nonsynaptic varicosities for biogenic amines in brain [7–9]. The presence of nontraditional synapses may be specific to certain brain regions [10], indicating that these biogenic amines may function both at conventional synapses and nontraditional ones. Further, in different brain regions the extent to which VT is involved in, for example, dopaminergic transmission may vary [11].

Consistent with the view of diffusion of transmitter to act at distant, nonsynaptic receptors is the realization that channels between cells are of sufficient width to allow the passage by diffusion of neuroactive compounds [12]. Although fraught with a variety of assumptions, it has been estimated that DA can diffuse at least 10 μm and 5HT 20 μm from its release site in brain tissue within one half-life [13,14], distances that would permit action at extrasynaptic receptors. Also, in a series of elegant investigations, Wightman and his colleagues [13–15] showed the concentration of either DA or 5HT in ECF to be directly proportional to the number of electrical pulses in an electrical train, a result not consistent with the buffered diffusion that occurs with hard-wired transmission. Further, peak extracellular concentration of either transmitter after a single stimulus was not altered by uptake inhibitors, suggesting that the uptake process is not altering the efflux of these transmitters into the extrasynaptic space. As is discussed in the section on transporters, one explanation for such data is that the uptake sites are extrasynaptic.

If DA or 5HT can "escape" from the synapse and diffuse in ECF some distance from the synapse, is there any evidence that they will encounter appropriate receptors outside the synapse? There appears to be. Although certainly not conclusive, much has been made of, and considerable controversy has been generated by, the many observations showing a "mismatch" in brain between areas receiving very little innervation by a specific type of neuron yet having a high density of receptors for the particular transmitter [16]. For example, in rat cerebral cortex, only the $5HT_2$ receptor has a distribution that appears to match the regional and laminar density of serotonergic innervation [17]. More convincing, though, are studies carried out with electron microscopy which reveal receptor immunoreactivity outside of synapses. This has been found for both D_1 and D_2 dopamine receptors [18,19], $5HT_{1A}$ [20] and $5HT_{2A}$ [21] receptors.

The foregoing lends credence to the view that DA, 5HT, and perhaps NE can spill out from synapses to diffuse to distal sites in concentrations that may be sufficient to activate extrasynaptic receptors [13,14]. This issue and its neuropsychopharmacologic implications are highlighted in the sections dealing with the localization of transporters and their regulation.

III. BIOGENIC AMINE REUPTAKE AND TRANSPORTERS

It has been 40 years since the initial observation that tritiated NE could be taken up from blood into organs containing sympathetic nerves [22], due to an active transport process contained in these nerves. Further research revealed that the primary means of terminating synaptic activity of NE, DA, or 5HT was by these active transport processes. Key neuropsychopharmacological discoveries were that many antidepressants inhibited the uptake of NE and 5HT [23,24], whereas psychostimulants, such as cocaine and methylenedioxymethamphetamine (MDMA; "Ectasy"), blocked the uptake of DA as well as that of 5HT, and, for some of the drugs in this class, uptake of NE was also inhibited [25–27]. The inhibition of uptake was thought to be responsible for the efficacy of antidepressants, whereas the inhibition of DA uptake was linked to the euphoric and reinforcing properties of psychostimulants.

Although the uptake processes for these three amines had similar characteristics, the uptake of each amine is mediated by a specific protein termed a transporter. Furthermore, the transporter proteins were presumed to have a synaptic localization to account for the enhancement of synaptic transmission thought to occur when pharmacological agents inhibited the uptake process. In other words, reuptake (and diffusion) altered the magnitude, duration, and spatial domain of transmitter-induced receptor activation and, in so doing, modified neurotransmission. More recent work [28] has substantiated the idea that these transporters are the *key* cellular elements regulating the concentrations of biogenic amines in ECF. The cloning of biogenic amine transporters in the early 1990s [29–32] and the development of selective radioligands for them at about the same time permitted a range of studies not possible previously. These studies have begun to provide important information about transporter function and regulation that in some cases expands and amplifies our previously held concepts, but in other ways, fundamentally changes them.

A. Structure of Monoamine Transporters

The dopamine transporter (DAT), norepinephrine transporter (NET), and 5-hydroxytryptamine transporter (5HTT) are part of a family of neuronal plasma membrane transporters that include the monoamines and certain amino acids such as gamma-aminobutyric acid (GABA), glycine, and proline [33]. These three transporters share considerable structural homology. They are all Na^+ and Cl^- dependent, have 12 membrane-spanning domains, N- and C-termini located intracellularly and a large extracellular loop with glycosylation sites which may alter trafficking and/or function of the transporters. The extracellular and intracellular portions of the proteins have phosphorylation sites that likely contribute to the functional properties of the transporters. The DAT, NET and 5HTT are each believed to represent a single gene product [33]. Since they represent single gene products, this means that posttranslational or other intracellular regulatory mechanisms must play a role in regulation of the function of these transporters. Data, reviewed below, are starting to appear that support the theory that phosphorylation of transporters through a variety of protein kinases and phosphatases causes changes in their function and plays a role in the trafficking and incorporation of transporters into the plasma membrane.

B. Models of Transporter Function

Current ideas about the function of monoamine transporters have led to proposals that transporters may operate in at least two modes: (1) as an alternating access carrier [34], or (2) in a channel mode [35]. In the more standard alternating access carrier mode (Fig. 1) [36], the protein is first in a conformation such that the cotransported ions, Na^+ and Cl^-, and the substrate (e.g., DA, NE, or 5HT) bind to a cleft in the transporter that is open to the extracellular space. The transporter then converts to a form that is accessible to the intracellular space, allowing the cotransported ions and the substrate access to the cytoplasm. This internal-facing form releases the transported substances into the cytoplasm and then interconverts so as to expose the now empty binding sites to the extracellular environment. This is the transport cycle. In the case of the 5HTT [34], K^+ ion binds to the transporter protein when it is open to the cytoplasm and may facilitate the interconversion of the protein to the form that exposes binding sites to the extracellular space to reinitiate the transport process. According to this model, the rate of

influx of extracellular solute determines the rate of efflux of intracellular solute; i.e., influx and efflux rates are modulated equivalently. Data have been obtained recently with the 5HTT and the DAT showing independent modulation of inward and outward transport [38–40]. Thus, some features of transport seem inconsistent with the classical alternating access carrier model.

By contrast, in the channel mode, which is thought to be a low probability event, the transporter protein functions as an ion channel (Fig. 1). Evidence in support of the channel mode of conductance is that the 5HTT and NET have transmitter-activated currents that are not linked stoichiometrically to substrate movement [35, 36, 41–45]. For example, if charge movement were merely linked to coupled transport for the NET, then one would predict one charge/NE molecule. What has been found for the human NET expressed in cultured cells is 200 charges/NE molecule. Such data have been interpreted to mean that these charges are carried by the positively charged NE molecules and cotransported ions [41]. Moreover, when the transporter is in the channel mode, a single transport event carries many more NE molecules than would be predicted by the classic alternating access model [46]. One way this could occur would be if the transporter acted as a channel permitting bulk flow of substrate through the open pore. This behavior of the transporter may be explained by the existence of two gates, one directed intracellularly and one extracellularly. In the case of the alternating access model, the two gates open sequentially during the transport cycle to allow the exchange of ions and substrate between ECF and cytosol by alternating access to the cleft of the transporter [36]. On occasion, the "gates" on both the extracellular and cytoplasmic sides of the transporter open simultaneously, permitting bulk flow of substrate and associated ions through an "ion" channel. It seems possible that transporters are really combined carriers and channels.

One implication of transporters acting as ion channels is that it should be possible to develop drugs that change the probability of the transporter acting in the channel mode, akin to the effect of benzodiazepines at the $GABA_A$ receptor. Such drugs should markedly influence the effect of synaptically released transmitter. If, for example, some psychotic states are linked to excessive dopaminergic transmission, drugs that change the probability of the DAT acting in the channel mode might be effective in these states. Another interesting aspect of the realization of transport-associated currents is that it permits analysis of the effects

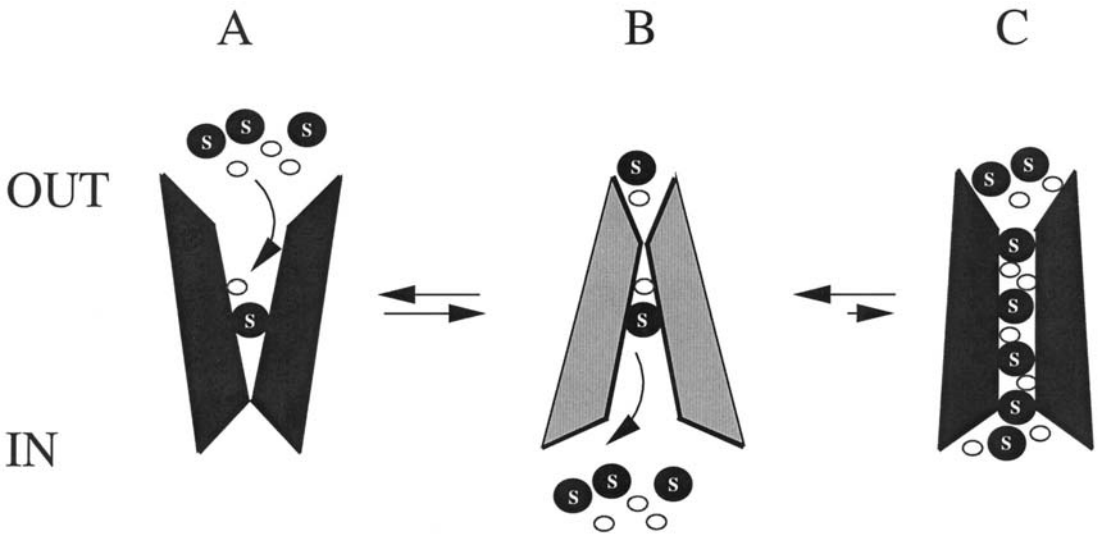

Figure 1 Schematic for biogenic amine transport in the alternating access and channel mode. In the alternating access model, the carrier has an aqueous lumen or cleft that exposes alternatively to the extracellular or intracellular environments. This transition state (A) ⇔ (B) results in transport of the substrate (S) and co-transported ions (empty circles) that is coupled stoichiometrically. The channel mode (C) is a low probability event, in which the ions and substrate move through the channel pore down their electrochemical gradients. The constrictions indicated on the cytoplasmic (A) or extracellular (B) domains of the transporter may be viewed as "gates", both of which are open simultaneously to form a pore (C). (Diagram courtesy of Dr. Aurelio Galli, Department of Pharmacology, University of Texas Health Science Center, San Antonio TX.).

of psychoactive drugs on such currents. These analyses may provide some explanation for differences in the pharmacological properties of "similar" drugs. For example, amphetaminelike compounds (including the neurotoxin 1-methyl-4-phenylpyridinium (MPP^+) acted like DA and caused transport-associated currents at DATs whereas cocainelike drugs (including methylphenidate) blocked such currents [47].

C. Electrogenic Processes

The DA, NE, and 5HT transport cycles involve cotransport of ions and therefore the processes are potentially electrogenic. One or two Na^+ ions and one Cl^- ion are cotransported, resulting in a net inward flux of positive charge [45,48]. In general, the electrogenic processes involved in transport may contribute substantially to the resting membrane potential of a given nerve terminal and affect not only the relative activity of the transporters but also other processes such as transmitter release. Flux of charges associated with substrate uptake has been demonstrated for all of the monoamine transporters [41,44,46,49]. Consistent with this is the finding that depolarization decreased whereas hyperpolarization enhanced DA uptake in xenopus oocytes [47]. It seems that the DAT is regulated similarly in a voltage-dependent fashion in the mammalian CNS [48,50–53].

The implication of this is that when DA neurons are depolarized, DAT will decrease its transporter activity, allowing for greater diffusion of DA to its receptors [54,55], perhaps distant from a synapse or varicosity (see above). By contrast, hyperpolarization of DA neurons would enhance uptake of DA so as to decrease its receptor-mediated effects. This scheme makes sense physiologically since, for example, situations that would call for depolarization-induced release of DA would "turn off" its inactivation mechanism (i.e., the DAT) in order to facilitate dopaminergic transmission. This regulatory process now appears likely for DA-containing neurons, and may also occur for the NET and 5HTT as well [41,46,56].

D. Anatomical Localization of Transporters

Although transporters were presumed to have a localization within the synapse, results of studies visualizing either the DAT or 5HTT by electron microscopy have revealed the presence of these transporters outside the synapse. For example, Zhou et al. [57] found the majority of 5HTTs to exist in small unmyelinated axons, suggesting 5HT uptake to be mainly extrasynaptic; they found also that 5HTTs on axons outside synapses were engaged in high-affinity uptake of 5HT. They speculated that 5HT can spill out from the synaptic cleft and that the 5HTT located just outside the synapse can take up 5HT near the synapse whereas axonal 5HTT takes up 5HT that has diffused to more distal sites. Evidence for extrasynaptic localization of 5HTTs has also been found in both the shell and core of the nucleus accumbens [21]. Similarly, Nirenberg et al. [58,59] visualized the DAT under electron microscopy in the substantia nigra, the dorsolateral striatum, and the nucleus accumbens, and found evidence in all areas for the DAT being outside of synapses.

The extrasynaptic localization of transporters, coupled with the idea of VT, means, for example, that DA may come into contact with NETs. This is of importance as it has been shown that the NET transports DA even better than NE [60]. By contrast, the DAT does not transport NE, but it has been demonstrated that 5HT can be taken up by the DAT in the striatum [61]. Much earlier work of Shaskan and Snyder [62] showed, using rat brain slices, that noradrenergic nerves could take up 5HT, albeit much less potently than they transported NE; however, their capacity to take up 5HT was much greater than that for NE.

Thus, transporter "promiscuity" coupled with VT could result, for example, in DA reaching NETs in sufficient concentrations so as to be taken up into noradrenergic nerves, or 5HT reaching DATs or NETs so as to be removed by these transporters. There is evidence in support of this concept. For example, in the ventral mesencephalon ^{3}H-DA can be taken up by serotonergic neurons and this effect is partially blocked by fluoxetine [63]. Results with in vivo microdialysis have shown that systemic administration of selective inhibitors of the NET raise DA in the prefrontal cortex [64], and local application into the nucleus accumbens of selective inhibitors of the NET raised extracellular levels of 5HT and DA in addition to NE [65]. Using in vivo voltametry, we also obtained evidence for the uptake of 5HT into noradrenergic nerves [66]. In the dentate gyrus of the hippocampus, where the density of NETs outnumbers 5HTTs by roughly 2:1 (unpublished observations), exogenously administered 5HT was taken up by both serotonergic and noradrenergic nerves. By contrast, in the CA_3 region, where 5HTTs outnumber NETs about fourfold (unpublished observations) no evidence of 5HT uptake into noradrenergic nerves was obtained.

Relevant to this issue is an interesting result of Bel and Artigas [67]. These investigators measured the con-

centration of 5HT in the ECF of the frontal cortex, obtained by microdialysis. Systemic administration of desipramine alone did not raise the concentration of 5HT whereas administration of fluoxetine did. However, when the concentration of 5HT was elevated by a dose of fluoxetine that produced a maximal effect, administration of desipramine was able to raise further the concentration of 5HT. One explanation for this result is that blockade of the 5HTT permitted 5HT to reach noradrenergic nerves where it was taken up by NETs located on them.

All the results cited above have certain limitations which make their extrapolation to the clinical situation problematic. Nevertheless, such results are consistent with data showing that depressed patients treated with selective inhibitors of the 5HTT show decreases not only in the concentration of 5-hydroxyindoleacetic acid (5HIAA) in spinal fluid but in the norepinephrine metabolite 3,4,dehydroxyphenylglycol (MHPG) as well; similarly, treatment of patients with selective noradrenergic uptake inhibitors results in decreases in both MHPG and 5HIAA in spinal fluid [68]. One implication, then, of VT and extrasynaptic localization of transporters is that administration of selective uptake inhibitors could facilitate the uptake of a specific transmitter into other types of nerves producing as yet unknown effects. Another implication is that administration of dual uptake inhibitors (e.g., imipramine, which inhibits the uptake of both NE and 5HT) could result in even greater diffusion of NE or 5HT from their release sites so as to reach receptors on targets that they would not if only one transporter was inhibited. The consequences of this are not clear, but this could be an important area for future research.

Thus, even though uptake inhibitors were developed for clinical use because of the idea that they would prolong "synaptic" transmission (and they may do so by inhibiting perisynaptic transporters), they may produce many other effects as well, both on the membrane potential and intracellular processes of the nerves containing the transporter that they inhibit and on other nerves at a distance from the site of transmitter release.

E. Regulation of Transporter Function

Because transporters for the biogenic amines both are critical in regulating the extracellular concentrations of these amines, and are key targets for a number of psychotherapeutic drugs, understanding how their function is regulated has come under intense scrutiny in recent years. Once the mechanisms for regulation of the biogenic amine transporters are understood, there is great potential for developing new classes of drugs for the treatment of disorders such as depression, mania, anxiety, schizophrenia, and drug abuse. It is becoming clear that transport of biogenic amines is not simply a constitutive property of synaptic membranes but a dynamically regulated component of aminergic signaling.

1. Acute Regulation of Transporter Function

Acute changes in transporter function can occur rapidly (within minutes). Consequently, it is unlikely that such changes are mediated via alterations in gene expression given that at least several hours are required for increases in transporter mRNA to translate into increased transporter expression in the plasma membrane [69]. Commensurate with the finding that transporters for the biogenic amines contain sites for protein phosphorylation by a number of kinases [33], several groups reported rapid changes in transport capacity following activation of cellular kinases. The most common observation was that activation of protein kinase C (PKC) led to a reduction in amine transport capacity [70–72]. There are also considerable data implicating a role for calcium, calmodulin, and other kinase-dependent as well as kinase-independent pathways in the acute regulation of transporter function [73–78]. The decrease in transport capacity ensuing PKC activation is due to a reduction in V_{max} with K_m remaining largely unaltered. The reduction in V_{max} is associated with a decline in the number of transport proteins in the cell membrane [75,79]. This seems to result from sequestration of the transporter for recycling rather than degradation [77,79–81].

New techniques have enabled researchers to track changes in the distribution of transporters within cells. The most extensive studies to date have been carried out in cell lines transfected with the DAT and have consistently demonstrated PKC activation to evoke internalization of this transporter [82–85]. The fate of internalized DATs remains under debate, with evidence for both recycling [85] and degradation [82]. Nevertheless, it is apparent that cell surface redistribution of biogenic amine transporters is a mechanism that contributes to regulation of extracellular levels of transmitter. Such cell surface redistribution has pharmacological significance. For example, Saunders et al. [86] showed that amphetamine, a substrate for the DAT, caused trafficking of the human DAT (*h*DAT) from the plasma membrane to the cytosol of cultured cells. Callaghan and coworkers [87] subsequently showed that cocaine exerted the opposite

effect, that is, mobilization of the *h*DAT from the cytosol to plasma membrane of cultured cells. Determining the pathways through which such psychotropic drugs are able to alter the distribution of transporters on the plasma membrane will have important ramifications for the development of new drug therapies for the treatment of numerous psychiatric disease states and drug abuse.

2. *Mechanisms for Trafficking of Biogenic Amine Transporters*

One way in which protein kinases can be activated is via presynaptic receptors. Apparsundaram and coworkers [79,81] demonstrated that activation of muscarinic acetylcholine receptors linked to PKC rapidly and selectively decreased the transport capacity (V_{max}) of the NET. In another study, Miller and Hoffman [88] reported that activation of A_3 adenosine receptors in cells increased 5HT uptake. This increase could be blocked by inhibitors of nitric oxide synthase and cGMP-dependent kinases, providing evidence for a nitric oxide–cGMP pathway in the acute regulation of the 5HTT.

In vivo studies have also provided evidence for receptor-mediated pathways in acute transporter regulation. For example, using high-speed chronoamperometry, Daws et al. [89,90] reported that antagonism of the $5HT_{1B}$ autoreceptor prolonged clearance of 5HT in rat hippocampus. Importantly, antagonism of the $5HT_{1B}$ autoreceptor in 5HTT knockout mice failed to alter clearance of 5HT, indicating that the presence of both proteins is required for this effect [91]. These observations are consistent with the idea that activation of $5HT_{1B}$ autoreceptors enhances 5HTT function. Similarly, blockade of the dopamine D2 receptor has been shown to inhibit clearance of DA from extracellular fluid [50,51], an effect that was absent in mice lacking the D2 receptor [52]. Whether autoreceptor regulation of transporter activity leads to transporter trafficking is unknown, as are the signal transduction pathways linking receptor to transporter.

Many signaling proteins, including receptors and ion channels, are modulated via direct protein phosphorylation, and there is evidence that this is also true for biogenic amine transporters [for a recent review, 72]. Blakely and colleagues [92] demonstrated that 5HT reduced phosphorylation of the 5HTT both under basal conditions and following PKC activation. These effects could be blocked by paroxetine, a selective serotonin reuptake inhibitor (SSRI), suggesting that the effects of 5HT were mediated by an action on the transporter and not on 5HT receptors. Ramamoorthy and Blakely [77] later showed that PKC-induced transporter internalization was reduced in the presence of 5HT [77]. The inhibitory effect of 5HT on phosphorylation of the 5HTT is due to its binding to the 5HTT and/or its translocation by it. This indicates that phosphorylation of the 5HTT occurs when it is in the plasma membrane, and further suggests that such phosphorylation serves as a signal for its trafficking and internalization. The ability of 5HT to suppress phosphorylation may be a mechanism to maintain high transporter function when extracellular levels of 5HT are elevated [71]. In contrast to the 5HTT, direct phosphorylation of the DAT does not appear to be involved in PKC-mediated regulation of DAT function [93]. Further, it now appears that phosphatases are also involved in regulating the state of phosphorylation of amine transporters [72,94], providing yet another approach to regulating the function of transporters in vivo.

3. *Long-Term Regulation of Transporter Function*

Several lines of evidence, including changes in transporter activity and/or expression in response to environmental perturbations (e.g., altered photoperiods) [73], fluctuations in hormone levels (e.g. corticosteroids, estrogen) [95–97], and as a consequence of aging [98,99], suggest that biogenic amine transporters also undergo long-term regulation.

Most relevant to the present review are the changes in transporter activity/expression observed in certain disease states and as a consequence of therapeutic intervention. For example, reductions in both 5HTT and NET binding have been reported in patients with depression [100–102], and significant reductions of 5HTT binding and DAT immunoreactivity have been observed in patients with Parkinson's disease [reviewed in 103]. Depressive disorders are commonly treated with selective inhibitors of 5HT and/or NE uptake. Pharmacologic inhibition of transporters occurs rapidly. However, maximal therapeutic benefit takes weeks to occur, so adaptive changes induced by such drugs on biogenic amine systems have been investigated extensively. Although much of this work focused on receptors and receptor-mediated responses [104], data on transporter function have also been obtained. Numerous studies have assessed the effect of chronic antidepressant treatment on the density of binding sites for the 5HTT and NET. The results have not been consistent [reviewed in 73,105]. Similarly, although

several studies have shown no effect of chronic treatment with antidepressants on mRNAs for the 5HTT and NET, others have reported increases and still others, no change [101,105,106]. Obviously, differences in the duration of chronic drug dosage and route of drug delivery, which inevitably exist in such in vivo studies, make firm conclusions regarding alterations in gene expression by chronic drug treatment difficult.

Using high-speed chronoamperometry, we [107] measured the ability of an SSRI, fluvoxamine, applied locally to the hippocampus, to inhibit uptake of exogenously applied 5HT in rats treated for 21 days by osmotic minipump with the SSRI paroxetine. The more constant level of drug in plasma obtained by administration via minipump may better model clinical administration of uptake inhibitors. Acute local administration of fluvoxamine did not inhibit uptake of 5HT in chronically treated rats (as it did in non-treated rats). This lack of an inhibitory effect of fluvoxamine may be due to a robust decrease in 5HTT-binding sites in paroxetine-treated rats [107]. Pineyro et al. [108] also found chronic administration of paroxetine by minipump to cause a decrease in 5HTT function and density. More recently, we [109] showed that the time course for decreased 5HTT function following chronic treatment of rats with an SSRI was gradual. This functional decrease was paralleled by a decrease in 5HTT density but not by mRNA levels for the 5HTT. These data suggest that posttranscriptional events mediate changes in 5HTT function caused by long-term administration of SSRIs. One implication of these data for the clinical setting is that antidepressant-induced decreases in 5HTT density may need to reach a "critical" level before therapeutic benefits are seen. In keeping with this, Alvarez et al. [102] reported that platelet 5HTTs were decreased in patients treated with fluoxetine. Thus, if transporter density can be reduced more rapidly, perhaps clinical improvement can be accomplished in a shorter time frame.

F. Genetic Knockout and Polymorphisms of Biogenic Amine Transporters

Perhaps the most striking demonstration of the importance of transporters in regulating extracellular fluid concentrations of biogenic amines comes from studies using transporter deficient mice. In vivo studies using voltametric recording techniques show that homozygous 5HTT knockout (KO) [91], DAT KO [110], and NET KO [111] mice have a profoundly reduced ability to clear 5HT, DA and NE, respectively, from ECF. However, in most cases, clearance remains more rapid than simple diffusion would predict. This implies that other mechanisms must compensate, at least to some extent, for the loss of transporter. One possibility is that transporters other than the specific transport protein for a given biogenic amine are able to compensate. As has been discussed, transporters for the biogenic amines exhibit some "promiscuity" for transmitters. In addition, the presence of nonneuronal monoamine transporters, such as the extraneuronal monoamine transporter and organic cation transporter 2 (OCT2), in brain [112] may also account for clearance of biogenic amines from ECF in 5HTT-, DAT- and NET-deficient mice. Indeed, OCT2 is reportedly increased in the brains of 5HTT KO mice [113] and may represent an adaptive response to the loss of 5HTT.

The advent of transporter-deficient mice and their altered responses to drugs as well as inherent differences in amine levels, receptors, and behavior [28,111,114], prompted researchers to look for genetic variants in transporter proteins that may predispose to psychiatric disorders. Variants have now been uncovered in the promoter region of the gene encoding the 5HTT that alter mRNA and protein expression both in vitro and in vivo. An association between these variants and a number of disorders, including anxiety, affective disorder, autism, and alcoholism, have been reported. Such associations include a predisposition for the disorder and altered sensitivity to drugs used to treat the disorder [115–118]. Clearly, this is an area for active research which should lend important insights into the underlying etiology of such disorders and improved treatments for them.

G. Implications for Neuropsychopharmacology

Our changing views on modes of neurotransmission (hard-wired vs. VT) and on transporter function (carrier vs. channel mode), together with the marked advances in our understanding of transporter regulation, have paved the way for the development of new drugs. For example, drugs that cause immediate activation of certain protein kinases (or inhibition of certain phosphatases) may speed or enhance the therapeutic efficacy of current antidepressant treatments by either (1) causing a rapid reduction in the number of active transporters at the plasma membrane (e.g., through sequestration) and/or (2) bringing about more rapid changes in transporter gene expression. Likewise, the development of drugs that alter the probability of transporters acting in channel mode, or of drugs that

allow greater diffusion of transmitter from their release sites, is also an area for active research because of their therapeutic potential. In addition, understanding the regulation of transporters and the consequences of VT may result in determination of the mechanisms that underlie the reinforcing properties of cocaine, amphetamine, and other drugs of abuse.

Although our understanding of the role of transporters and of diffusion in regulating monoaminergic neurotransmission has increased tremendously in recent years, these remain more "classical" concepts. More recently the idea of peptidergic regulation of monoaminergic neurotransmission has emerged. In particular, there is new evidence that neuropeptide receptors may be novel targets for antidepressant and anxiolytic drugs. Therapeutic efficacy induced by antagonists of certain neuropeptide receptors is thought to be due to changes in noradrenergic and serotonergic activity. Because of this it is timely to review our current understanding of neuropeptide regulation of monoaminergic neurotransmission.

IV. NEUROPEPTIDE MODULATION OF MONOAMINERGIC NEUROTRANSMISSION

In the late 1970s and early 1980s, much fanfare hailed the emergence of a "new" class of brain neurotransmitter, the neuropeptides. Every year, more peptides were isolated, identified, quantified, and mapped anatomically in the brain. There was much excitement and anticipation that this era would generate a new understanding of neurotransmission and regulation of brain function. A noteworthy feature of neuropeptides that emerged from this period of research is that they seem invariably to be colocalized in the same nerve terminals with other neurotransmitters, including the monoamines [119], essentially forcing an obligatory inteaction between the two transmitter classes. Nonetheless, the nature of this interaction and the contexts in which it occurs must be determined by the complement of receptor subtypes expressed by the postsynaptic target neuron, and also by the differential release characteristics of the colocalized peptidergic and monoaminergic neurotransmitters.

A. Neuropeptides Are "Slow" Modulatory Transmitters

The general characteristics of peptide neurotransmission differ in many important respects from those of monoamines [see 120]. Unlike monoamine transmitters, which are derived from a single amino acid by enzymatic synthesis, neuropeptides are small proteins comprising a chain of several amino acids. As such, they are synthesized in the cell body by ribosomal translation of messenger RNA. Like other proteins, they are usually synthesized first in the form of a precursor protein, which is then further cleaved and modified before the peptide is shipped via axonal transport to the nerve terminal. There it is incorporated into large dense-core synaptic vesicles [121]. Thus, unlike the relatively rapid changes that can be induced in the rate of enzymatic synthesis of monoamines directly in the nerve terminal, regulatory induction of neuropeptide synthesis is a slow process, requiring hours or even days for the activation of gene expression, *de novo* protein synthesis, and axonal transport before any change in releasable peptide becomes available to the nerve terminal.

The localization of peptide transmitters in large, dense-core synaptic vesicles, as opposed to the small, clear vesicles in which monoamines are found, also confers unique release characteristics on peptides. These large vesicles are situated father from the so-called active zones [122], than are the small clear vesicles. Thus, they are farther from vesicle docking and release sites, and farther from calcium entry sites, rendering them less sensitive to low levels of electrical activity in the nerve terminal. The practical implication of this is that monoamines show a fairly graded relationship between firing rate in the presynaptic fiber and the amount of neurotransmitter released into the synapse, whereas neuropeptides are preferentially released under conditions of intense activation or burst firing [123]. Thus, whatever regulatory interactions the peptides may have with monoamines, they are likely to occur preferentially under conditions of intense activation of the neuron in which the two transmitters are colocalized.

Unlike the monoamines, for which reuptake by specific transporters is so important to the regulation of synaptic effects, no such reuptake transporters have been identified for neuropeptides in the brain. Rather, termination of the synaptic action of peptides appears to depend upon bulk diffusion and extracellular enzymatic degradation by general peptidases, which can be located at some distance from the synapse [124]. Thus, not only is the synthesis and release of neuropeptides slower than that of monoamines, but the termination of action is slower as well. Moreover, the lack of transporter-mediated reuptake means that peptides are likely to engage in VT, as defined earlier in this

chapter. This is supported, perhaps even more strongly than with monoamines, by frequent "mismatches" between the distribution of peptide-containing nerve terminals and appropriate postsynaptic receptors in the brain [reviewed in 125]. These characteristics have all led to the general view that neuropeptides function primarily as neuromodulators, altering effects exerted by other neurotransmitters on the activity of target brain circuits.

The monoamines have themselves been described as serving neuromodulatory functions in the brain [126]. Thus, the corelease of neuropeptides and monoamines during stress, arousal, reward, etc., confers a much higher level of complexity on this modulatory process. With increasing neuronal activity, proportionately more monoaminergic transmitter is released, and its modulatory effect is presumably increased accordingly. However, at some threshold level of activity, the release of a neuropeptide cotransmitter may be progressively recruited. The peptide may itself have modulatory effects on the same target, or it may modify the presynaptic release or the postsynaptic effects of the monoamine with which it has been coreleased.

B. Potential Modes of Interaction Between Neuropeptides and Monoamines in the Brain

There are several ways that neuropeptides and monoamines may interact in the brain. They can be colocalized and coreleased onto common targets, whereby they may exert their respective postsynaptic modulatory effects independently, cooperatively, synergistically, or in opposition, depending on the physiological context and the complement of receptors expressed by the post-synaptic target neuron (see reviews [120,127] for general discussions of colocalized transmitter interactions). Alternatively, they may originate in different afferents that converge onto a common target. In this case, the possibilities for postsynaptic interaction are the same, though independent activation of the different afferent pathways allows for more context specificity in the modulatory interaction. Finally, peptidergic neurons may innervate monoaminergic neurons or terminals, thereby affecting the firing rate or release of the monoamine transmitter in its target region.

The recent development of new tools and techniques, together with the novel application of established approaches, is just now providing both the means and the mindset for making substantive progress in understanding the functional interaction between brain neuropeptides and monoamine transmitters [128]. As this understanding progresses, we are forging a richer understanding of the potential contribution of neuropeptides, and their interaction with monoamine transmitters, in the development or treatment of affective disorders, including depression and anxiety [see reviews 129–133].

C. Substance P Antagonists as Novel Antidepressant/Anxiolytic Drugs

Substance P (SP) and its receptors are present in high concentrations in many forebrain limbic areas that have been implicated in affect and anxiety, including the hypothalamus, septal region, amygdala, and bed nucleus of the stria terminalis, as well as the periaqueductal gray, locus coeruleus, and raphe nuclei [134]. As for its relationship with NE and 5HT, the monoamines most implicated in the etiology and treatment of affective disorders, the substrates exist for many potential modes of interaction between these transmitters. Substance P terminals and receptors overlap those of both NE and 5HT. Likewise, SP fibers innervate serotonergic neurons of the dorsal raphe and noradrenergic neurons of the locus coeruleus. Finally, SP is colocalized with 5HT in ascending projections innervating the limbic forebrain in humans, other primates, and certain other species such as guinea pigs, though apparently not in rats or mice [135].

Surprisingly, whereas SP itself exerts primarily excitatory effects, blockade of SP receptors enhances both noradrenergic and serotonergic activity, most likely through a process of multisynaptic disinhibition [136]. Thus, SP antagonists may have an effect on monoaminergic transmission that is similar, at least acutely, to the effect of classical antidepressants that block monoamine reuptake. In preclinical behavioral assays, systemic or intraventricular administration of a SP antagonist attenuated anxietylike behaviors [137]. In animal models of anxiety- or depressive-like behavior, SP antagonist administration had effects similar to those of established antidepressant and antianxiety compounds [138]. These experiments on the affective response to SP antagonist administration culminated in a clinical study of the antidepressant and antianxiety efficacy of a centrally active SP antagonist in depressed patients [137]. The results of this study showed that the SP antagonist exerted both antidepressant and antianxiety effects, comparable to those of the SSRI paroxetine. This study showed great promise for the establishment of a novel antidepressant agent. Caution is necessary, though, until these clinical results

can be replicated. Nonetheless, should SP antagonists ultimately prove useful and efficacious against depression, their targeting a specific neuropeptide that interacts in a novel way with monoamines opens a new possibility for more widely effective, more efficient, or faster treatment of affective disorders.

D. Interaction of Neuropeptide Y and Galanin with Norepinephrine in Modulating Behavioral Reactivity to Stress

Two neuropeptides are prominently colocalized with NE in the locus coeruleus (LC)—neuropeptide Y (NPY) and galanin (GAL). Galanin is expressed in nearly all noradrenergic neurons in the locus coeruleus [139]; thus it likely serves as a cotransmitter in the many limbic forebrain sites innervated by the LC. By contrast, NPY is found in a much smaller proportion of noradrenergic neurons in the LC, but is extensively colocalized with NE in medullary noradrenergic neurons [140].

The central nucleus of the amygdala and the bed nucleus of the stria terminalis, two closely related components of the extended amygdala, are targets of dense noradrenergic innervation, and both have been implicated in fear and anxiety [141]. In a recent series of experiments, we demonstrated that the stress-induced release of NE in these regions facilitates the expression of anxietylike behavioral responses to acute stress [142,143]. This is consistent with the role proposed for NE in modulating affective components of the stress response, including vigilance, arousal, and anxiety [144–146]. By contrast, administration of NPY into the central nucleus exerts distinct anxiolytic effects [147–148], while local administration of NPY antagonist drugs into LC target regions can be anxiogenic [149].

Much like the autoinhibitory effects of NE acting on presynaptic alpha-2 adrenergic autoreceptors, it has been shown that NPY also acts on presynaptic NPY autoreceptors, reducing the release of both NE and NPY [150,151]. In addition, NPY receptors located on noradrenergic cell bodies inhibit the activity of these cells [152,153]. Thus, corelease of NPY with NE invoked when high levels of activity have been stimulated in noradrenergic neurons, may attenuate the anxiogenic effects of NE released in the limbic forebrain, exerting direct anxiolytic effects postsynaptically while at the same time acting presynaptically to inhibit the further release of NE.

Even more than NPY, galanin is extensively coexpressed with NE in the LC [139]. Like NPY, NE neuronal activity is also inhibited by galanin [154,155], and galanin receptors located on NE terminals, which may function either as postsynaptic heteroreceptors or as inhibitory autoreceptors that limit the release of galanin from those terminals, also inhibit the release of NE [151]. In a recent series of studies, we have shown that galanin exerts an anxiety-buffering effect in the central amygdala, attenuating the anxiogenic effects of acute stress that we showed were attributable to NE [156]. In this case, however, the galanin-mediated anxiolytic effect was elicited specifically when stress-induced activation of the noradrenergic system had been accentuated by prior administration of the autoreceptor antagonist yohimbine [158,159].

Along with this context specificity in the CeA related to the level of activation of the noradrenergic system, additional studies revealed that the functional interaction between NE and galanin in other regions of the limbic forebrain during stress were more complicated. In the bed nucleus, acute stress also induced NE release to facilitate anxietylike behavioral responses, but in this region, galanin facilitated these same behavioral responses [156,157], thus acting in the same direction as NE. Moreover, this facilitatory effect of galanin in the BST did not require prior treatment with yohimbine, as did the anxiolytic NE-buffering effects of galanin in the CeA. This is perhaps due to the fact that the major source of noradrenergic innervation in the bed nucleus arises from caudal medullary noradrenergic cell groups rather than the LC. Unlike the LC, these other noradrenergic cell groups do not show a high degree of galanin colocalization [139]. Thus, anxiolytic effects in the CeA may have originated from the corelease of galanin and NE from noradrenergic terminals, while anxiogenic effects in the bed nucleus may have originated from the activation of galanin-synthesizing neurons within the nucleus itself.

These neurons may themselves be targets of noradrenergic innervation [160], which would explain why their activity was elicited specifically in response to stress. Thus, depending on the level of activation of the noradrenergic system, the specific physiological context in which that activation occurred, and the specific brain region involved, galanin could either act in concert with or oppose the stress-induced behavioral effects of NE. Likewise, any drug that mimicked or blocked the effects of galanin in the brain could have anxiogenic, anxiolytic, or mixed effects depending on the context and the circumstance by which the behavioral response had been elicited.

Such complex interactions between transmitters of different classes may be subject to modification by a number of factors, including prior exposure to stress, chronic drug treatment, or genetic predisposition. Given the nature of this interaction, it is clear that drugs that affect monoaminergic neurotransmission could induce regulatory changes in both neuropeptide and monoaminergic functions, disrupting or resetting the delicate balance between these modulatory transmitters, either contributing to or interfering with their clinical effects.

In summary, then, the past decade has witnessed tremendous increases in our understanding of the complexity of the process of monoaminergic transmission and its regulation. This increased understanding has not yet been translated into substantial therapeutic advances but clearly has the potential to do so.

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4

Developments in Psychiatric Neuroimaging

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I. INTRODUCTION

The scientific inquiry into the human mind seeks to decode how brain structure and activity result in the vast range of cognitive and emotional processes each of us experiences. It also attempts to identify the anatomical and functional correlates of abnormal mental activity, as represented in the neurological and psychiatric disorders. In this perspective, neuroimaging has effected a radical change on the study of the connection between brain and mind.

Early investigations of brain function had to depend on indirect approaches. Lesion experiments on animals and postmortem clinicopathological correlations were the usual methods to investigate cerebral function, with obvious limitations. It was only at the end of the 18th century that the idea that different structures of the nervous system could perform distinct functions began to predominate. During the 19th century, the notion of functionally distinct cortical areas became well established, initially with the controversial works of Gall and the Phrenology school, and later with the discovery of the association of frontal cortex lesion and aphasia by Paul Broca [1]. Afterward, the conceptualization of the neuron, the discovery of specific cytoarchitectonics of different cortical areas, and the electrophysiological experiments on animal and later human cortex have set up the theory of cortical localization of mental functions as the mainstream scientific framework for brain investigation. Event-related potentials and single-neuron recording, 50 years ago, provided further experimental support for cortical localization. But it was only in the early 1970s, with the advent of X-ray computed axial tomography (CT), that it was possible to visualize the brain parenchyma in vivo [2]. Since then, the field of neuroimaging has undergone astonishing developments, and these new methods have rapidly become the most powerful tools to contribute to the understanding of neural organization and mechanisms underlying the mental phenomenon.

The present chapter does not intend to be an exhaustive review of the various neuroimaging techniques currently in use. The specific findings of neuroimaging studies in various psychiatric disorders will be presented in other sections of this book. In this chapter, we have focused on the imaging methods of highest relevance for investigations of the neural basis of behavior, providing an overview of the physiological rationale underlying each method. Each available method

assesses a specific feature of the intricate cerebral machinery, with its typical resolution on the spatial and temporal domains, and characteristic methodological limitations. For didactical reasons, we grouped the various techniques into three groups: structural neuroimaging, including the methods designed to explore brain anatomy and structure; chemical neuroimaging, for the methods dedicated to evaluate cell metabolites, neurotransmitters, and receptors in the brain; and functional neuroimaging, encompassing the techniques that evaluate cerebral perfusion, metabolism, and neuronal activation.

II. STRUCTURAL NEUROIMAGING

Before the first CT studies, only highly invasive radiological approaches could be utilized to evaluate brain structural abnormalities in human subjects. Pneumoencephalography, which consisted of an X-ray after air injection into the encephalon through a lumbar puncture, was utilized during the first half of the 20th century to examine the ventricular system. This technique has provided the first in vivo indications of enlarged ventricles and cortical atrophy on schizophrenic patients [3]. The advent of CT yielded a booming interest on structural neuroimaging of psychiatric disorders. Measurements of ventricular dilatation and cortical and cerebellar atrophy could then be performed in several psychiatric disorders [4]. But the evaluation of specific brain structures was still challenging, due to the limited contrast between gray and white matter observed in the CT images. Also, artifacts on the posterior fossa were relatively common owing to dense bone structures surrounding this region, which made brainstem and cerebellum more difficult to evaluate with CT scans. Most of the shortcomings in the earlier structural brain imaging studies were overcome with the advent of magnetic resonance imaging (MRI).

The first commercially available MRI scans appeared in the early 1980s, but the phenomenon of nuclear magnetic resonance has been under study since the 1930s, through the landmark works of the American physicist Isaac Rabi [5], who received the Nobel Prize in physics in 1944. Structural MRI is one of the several brain-imaging technologies that explore the magnetic properties of the atomic nucleus. The MR method is based on the property of some atoms, whose nuclei present an odd number of either protons or neurons, to posses "spin"—i.e., a net magnetic charge, like a small bar magnet. Only the atoms that have this property will be "visible" through nuclear magnetic resonance (NMR). Some biologically relevant examples include ^{1}H, ^{31}P, and ^{23}Na. Also, ^{7}Li and ^{19}F can be detected using NMR. Although present in negligible concentrations in the human brain, these atoms have important pharmacological relevance. On the other hand, atoms such as ^{12}C and ^{16}O are invisible to NMR.

The atoms visible to NMR present a random distribution of the orientation of their nuclear magnetic moment when no external magnetic field is applied. However, when under an external magnetic field (B_0), the nuclei of these atoms tend to align with this field, in the same (lower energy) or the opposite direction (higher energy) (see **Fig. 1**). This is the first step in the acquisition of MR images: to immerse the brain in a strong magnetic field, usually ~ 0.5–3 Tesla. For comparison, 1 Tesla is $\sim 20{,}000$ times the Earth's magnetic field. Under the action of the magnetic field, the nuclei will spin and generate a movement of precession (see **Fig. 2**), whose frequency is characteristic for each

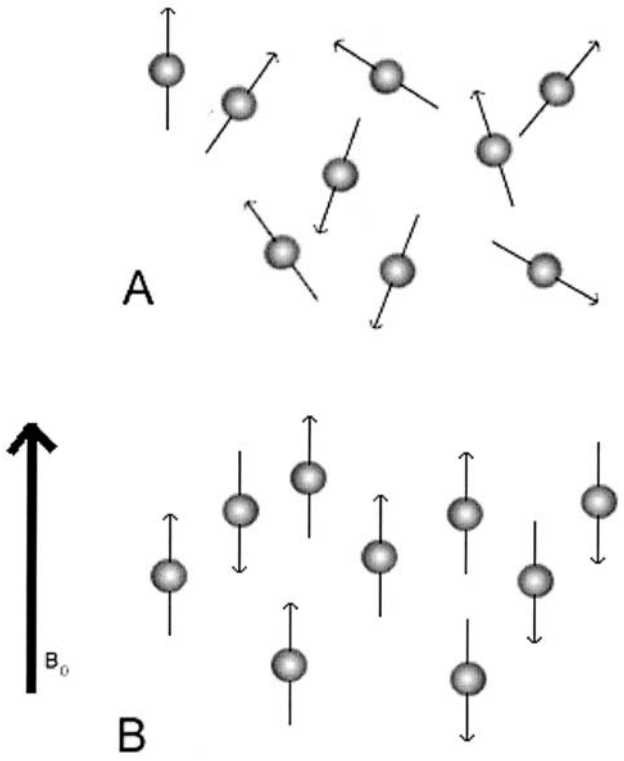

Figure 1 The nuclei of the individual atoms have a random distribution of their magnetic moment (A), with no net direction. When an external magnetic field B_0 is applied (B), all spins align either against or on the same direction of the field.

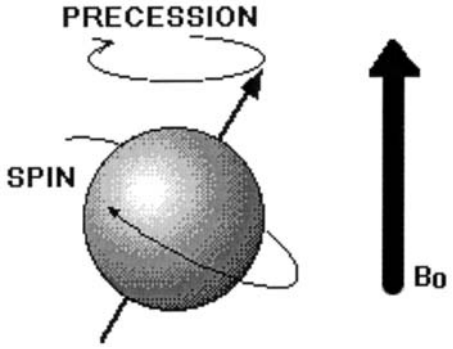

Figure 2 Spinning and precession.

atomic nucleus, and is proportional to B_0 strength. The next step is to expose these nuclei to a short-duration electromagnetic field (B_1, orthogonal to B_0), usually in the radio frequency range. This pulse excites the nuclei, disturbing the previous equilibrium state and inducing a transient phase coherence among the nuclei. This resonance can then be detected as a radio signal through a receiver coil. After turning off B_1, all nuclei return to equilibrium, i.e., from high-energy (excited) to low-energy (equilibrium) state. This process is associated with exponential loss of energy to surrounding nuclei; the time required for the magnetization to return to 63% of its original value is called T1. Since the process is exponential, the spins are usually completely relaxed after 3–5 T1 times. When returning to equilibrium, spins with high and low energy can also exchange energy without loosing it to surrounding nuclei. This phenomenon is termed spin-spin relaxation, and it is related to exponential loss in the transverse magnetization. Similarly, the time required for 63% of transverse magnetization to subside is called T2. For pure water, T1 and T2 are practically the same, around 2–3 secs. However, for most biological materials T2 is far shorter than T1. By varying parameters such as the repetition time or echo time of the radio-frequency signal, it is possible to acquire T1- or T2-weighted images, and consequently obtain distinct information from the biological tissues under analysis. Of course, this is a very simplified explanation of the mechanisms underlying the NMR phenomenon. Nonetheless, it is important to keep in mind that NMR can provide a varied range of information in a noninvasive fashion, from hemodynamics to cell chemistry. In the case of MRI, the resonance of large amounts of ^1H in the brain provides high-quality structural image, with spatial resolution of $< 1\ mm^3$, allowing the identification of small brain structures.

Most morphometric studies with MRI utilize an approach known as region of interest (ROI). Basically, the area of a specific brain structure is manually "traced" directly in the image, and the final volume is estimated from the number of slices that intersect the structure under study (see **Fig. 3**). Usually this is done in a blind fashion; i.e., the researcher is not aware of the diagnosis of the subject whose brain MRI is being evaluated. Standardized protocols defining the boundaries of the structure are utilized, which enables reliable reproduction of these methods among different researchers. In some cases, semiautomated procedures can be used to detect the border of particular brain structures, or to derive three-dimensional volumes from the tracings. Also, algorithms are used to segment the brain into gray and white matter and CSF, allowing more specific measurements. ROI-based morphometry has provided extremely important contributions over the past several years, allowing the study of relationship between structural anatomy and psychopathology.

However, this method presents some limitations. Certain arbitrariness is necessary to set the boundaries of structures that do not have well-defined edges. Another limitation of ROI-based analysis is that struc-

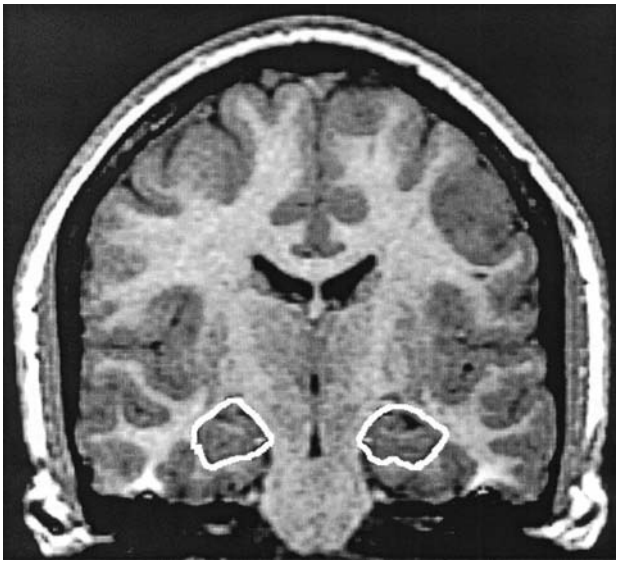

Figure 3 Example of a structural MRI, with the hippocampus traced bilaterally in a coronal slice. In the region-of-interest analysis, an anatomical structure is usually traced in several slices, and the volume is derived from the sum of the areas multiplied by the slice thickness. For the hippocampus, semiautomated procedures are utilized to include in the analysis only the pixels identified as gray matter.

tures with different shapes can have the same volume. Thus, if the shape of an anatomical structure is different between patients and controls, but with similar final volumes, no differences between the groups will be detected. As an attempt to minimize these shortcomings, new experimental designs have been developed. For instance, it is possible to compare the content of gray matter between two groups of subjects in a voxel-by-voxel basis. Voxel is the three-dimensional graphic unit of an MR image, and through a specific mathematical approach, it is possible to examine the whole brain and identify areas that present a lower density of gray matter (voxel-based morphometry), or to compare the relative position (deformation-based morphometry), or local shape (tensor-based morphometry) of certain brain structures among different groups [6]. These statistical parametric methods have been used for functional data, and now are being validated for structural neuroimaging studies.

Nonetheless, a basic shortcoming underlies most of these morphometric methods: the links between the volume of a specific structure and the pathophysiology are still tentative. Data from other neuroimaging methodologies should be considered together to provide a better insight into the relevance of a volumetric finding. The cellular abnormalities underlying an atrophic cortex, for instance, can only be assessed directly through postmortem studies, or indirectly with MR spectroscopy (see below). Also, brain areas that are dysfunctional, but that maintain the same final volume and gray matter density of a healthy structure, will not be identified with morphometric studies, only with functional neuroimaging approaches. Moreover, typical MR images have a limited capacity to discriminate distinct white matter tracts. A very interesting and complementary morphometric approach, diffusion tensor imaging (DTI), has provided new possibilities to examine white-matter fibers. This MR methodology is based on the fact that water molecules inside axons have restricted diffusion; i.e., they diffuse faster in the direction of the axonal fibers than perpendicular to them. This property can be used to map white-matter tracts in vivo, and study the integrity of the connections among different brain areas [7].

The clinical application of MRI in psychiatry is limited, in most cases, to rule out neurologic abnormalities that might be responsible for the psychiatric symptoms, such as stroke or brain tumors. Even with the vast advances in structural neuroimaging observed in recent decades, it is still not possible to individually identify subjects with psychiatric disorders based on brain imaging. Typically, there is a considerable overlap among the structural measurements of patients and healthy controls, even when the patients, as a group, present a statistically significant difference from the control group. Nevertheless, anatomical abnormalities identified with structural neuroimaging studies are helping to develop, in parallel with other neuroimaging approaches, integrated models of pathophysiology of mental disorders.

III. CHEMICAL NEUROIMAGING

Abnormalities in the signaling among neurons have been implied in virtually every neurobiological model for psychiatric disorders. The mechanism of action of drugs with profound effects on behavior, such as antidepressants and antipsychotics, has supported the idea of an imbalance in specific neurotransmitter systems in mental illnesses, e.g., the dopamine hypothesis in schizophrenia, and the monoaminergic theories in depression. However, most of the studies on neurotransmitter functioning were restricted to postmortem brain tissue or peripheral blood cells. This picture has changed drastically with the advent of positron emission tomography (PET) and single-photon emission computed tomography (SPECT). These techniques can provide in vivo anatomically localized information about several parameters of neural transmission, metabolism, and pharmacology.

PET and SPECT are imaging techniques that can quantify and localize biologically relevant molecules, marked with a radionuclide. The brain uptake of the molecule of interest can be ascertained by measuring the amount of the radiotracer in each specific brain region. PET and SPECT are based on different properties of radioactive decay. Basically, in PET the radiotracer decays emitting a positron, which collides with an adjacent electron. This leads to the annihilation of both particles and to the release of two gamma rays (photons) which exact opposite directions [8]. The radiation detector surrounding the brain can detect this coincident emission (see **Fig. 4**). A computer registers that the marked molecule was present at some point along this imaginary line, and later rebuilt a three-dimensional map of the amount of radiotracer for the whole brain. On the other hand, in SPECT, the radiotracer absorbs an electron when decaying, which results in an unstable nucleus that emits a single gamma-photon in this process [8]. The detector, also called *collimator*, rotates 360° around the subject's head, and later translates this information into a picture of the distribution of the radionuclide in the brain

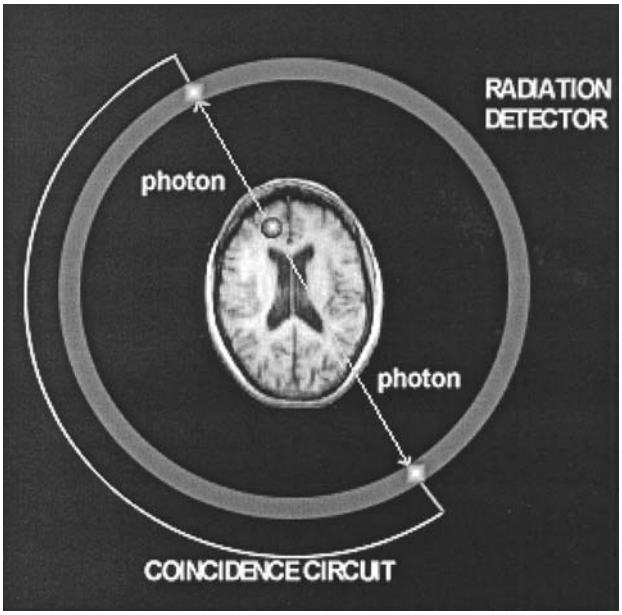

Figure 4 Schematic representation of a PET scanner.

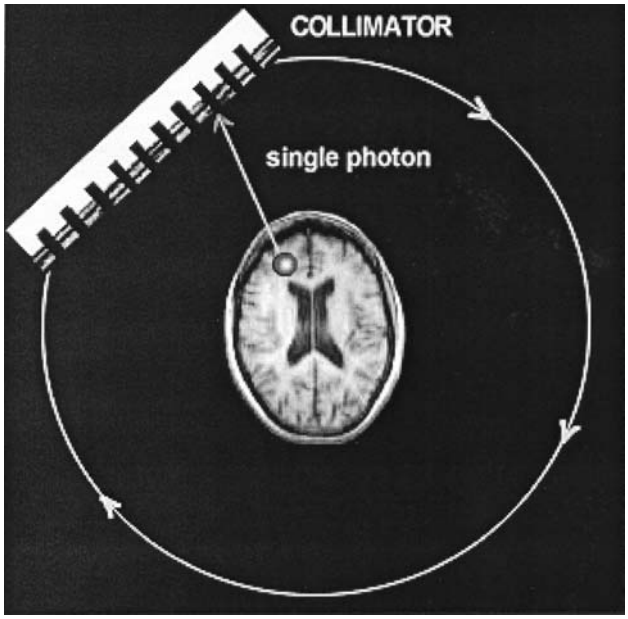

Figure 5 Schematic representation of a SPECT scanner.

(**Fig. 5**). Owing to these differences in the physical principles, PET and SPECT utilize radiotracers with distinct intrinsic properties, with PET usually providing better spatial resolution than SPECT.

Several aspects of in vivo neurochemical functioning can be assessed with either PET or SPECT. Numerous radiotracers have been developed to identify specific targets in the brain, including neurotransmitter synthesis and release, receptor occupancy and density, monoamine transporters and metabolism [9–11]. Dopaminergic, serotonergic, GABAergic (see **Fig. 6**), opioid, and cholinergic pathways have been studied through PET and SPECT radiotracers in several neuropsychiatric disorders; extensive reviews of available findings can be found elsewhere [12]. Also, PET and SPECT receptor studies have proven informative about the in vivo brain actions of several medications during clinical treatment. For instance, typical and atypical antipsychotics present a distinct pattern of occupancy of dopaminergic and serotonergic receptor subtypes [13,14]. These studies are particularly relevant to the investigation of links between side effects and response to treatment and receptor occupancy, and can aid in the development of new drugs.

Although the amount of radiation in the radiotracers currently utilized for research is minimal, the utilization of radioactivity represents a disadvantage of PET and SPECT techniques, since it limits the number of sessions in which a subject can participate. Better spatial resolution and a greater diversity of available radiotracers make PET a more attractive methodology than SPECT for in vivo brain investigations in neuropsychiatry. However, PET radioligands typically have very short half-lives, so a cyclotron located near the PET scanner is required to the production of these tracers. This makes PET scan a very expensive technology: about US$5 million is necessary to install a PET center, not including the costs related to the extremely specialized and multidisciplinary staff [15]. Since SPECT costs a fraction of PET, and is more widely available, it is expected that improvements in tracer development and technical complexity will result in further advances of SPECT studies in psychiatry.

Measures of brain chemistry can also be obtained through the phenomenon of nuclear magnetic resonance. As stated in the previous section, several different atoms have nuclei with magnetic moment and can therefore be visualized through NMR. For instance, in MRI the images are formed through the resonance of huge amounts of ^{1}H atoms in water and fat. However, ^{1}H is also present in several other metabolites, and the resonance frequency of hydrogen is different depending on the molecule it is found. This occurs because nuclear resonance frequency is influenced by the magnetic fields of the nearby electrons and nuclei; i.e., the molecular environment of a certain nucleus produces a resonance frequency for this nucleus that is slightly different than the resonance of the nucleus alone.

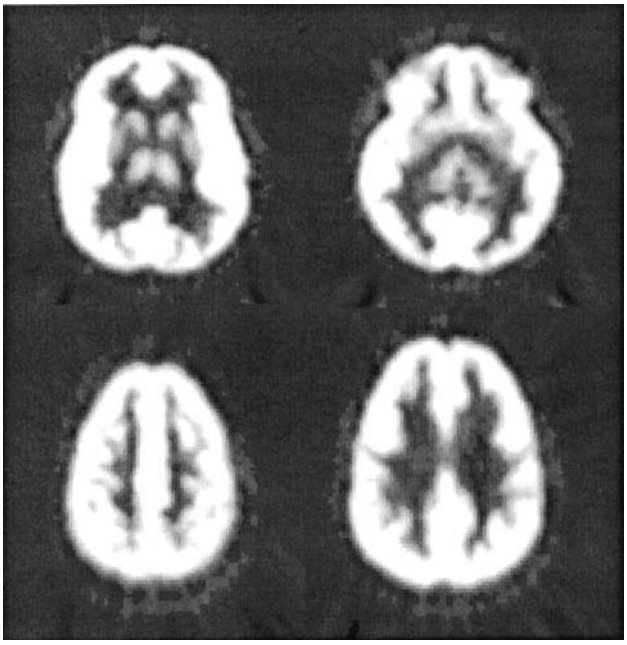

Figure 6 PET scan image showing the brain distribution of GABA A / benzodiazepine receptors using the radiotracer [^{11}C]-Flumazenil. Brighter areas indicate higher concentration of the tracer, and higher receptor densities.

This resonance is characteristic of each molecule, and can be represented in a scale called *chemical shift*, expressed in parts per million (ppm). For instance, the protons in water and fat exhibit two different frequencies, separated by approximately 3.5 ppm [16].

Several different ^{1}H-containing molecules will result in a spectrum of frequencies. This is the principle of magnetic resonance spectroscopy (MRS), a technique that has been used for decades in chemistry and physics to provide information about molecular structure and dynamics [17], and more recently has been employed in the study of in vivo brain chemistry. Given that different nuclei such as ^{1}H, ^{31}P, ^{23}Na, ^{13}C, ^{7}Li, and ^{19}F can be visualized in MRS, this technique allows measurements of molecules containing these nuclei in a noninvasive fashion, and without using radiation.

Since the concentration of most metabolites of interest in the brain is $\sim$10,000 times smaller than the concentration of water, the signal of these metabolites is much weaker. Therefore, MRS studies usually exchange spatial resolution for chemical information. Although MRS procedures are evolving toward improvements on spatial resolution, most studies still utilize a volume-of-interest (VOI) approach; i.e., voxels usually ranging from 1 to 8 mL are placed in relevant anatomical locations, and a spectrum is acquired from each VOI. A spectrum is a plot of intensity versus frequency, with each peak representing a different resonance frequency, i.e., different metabolites, whose concentrations can be estimated from the area under the peaks (see **Fig. 7**).

A varied range of chemical information relevant to research in psychiatry can be acquired from the in vivo brain with MRS [18]:

1. ^{31}P MRS studies enable measurement of pH, inorganic P, adenosine diphosphate (ADP) and triphosphate (ATP), phosphocreatine (PCr), phosphomonoesters, and phosphodiesters. Thus, information on cell membrane integrity and high-energy phosphate metabolism can be obtained.
2. ^{1}H MRS can quantify metabolites involved in neurotransmission (glutamate and choline), energy metabolism (PCr, creatine, lactate, and acetate), second messenger systems (myoinositol), membrane metabolism (phosphocholine and phosphoethanolamine), and neuronal viability (N-acetyl aspartate).
3. Pharmacokinetic and pharmacodynamic data can be obtained through ^{7}Li (brain lithium concentra-

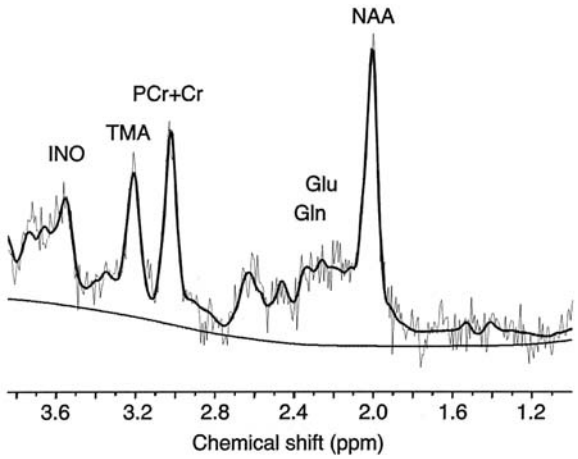

Figure 7 After the processing of the raw MRS data, a mathematical model allows the identification of the peaks corresponding to the ^{1}H resonance frequencies in each distinct molecule. The concentration of each metabolite is then derived from the area under the respective peak. In ^{1}H MRS, the resonance signal of water is suppressed to permit the quantification of other ^{1}H-containing metabolites. The most important metabolites assessed with ^{1}H-MRS are: N-acetyl aspartate (NAA), phosphocreatine + creatine (PCr + Cr), trimethylamines (TMA; commonly referred to as choline containing molecules, which mainly includes phosphorylcholine and glycerophosphocholine), myoinositol (INO), glutamate (Glu), and glutamine (Gln).

tion and distribution, and its correlation with outcome/side effects) and ^{19}F MRS (measurement of the brain concentration of fluorinated compounds, such as fluoxetine and fluphenazine).

4. Glucose metabolism and its relation with the glutamate/GABA cycle can be assessed with ^{13}C MRS [19].

Thus, MRS provides noninvasive measurements of a variety of chemical species *in vivo*, and has the potential to bring important contributions for the investigation of brain chemistry in neuropsychiatric disorders. However, the current MRS methodology still presents significant limitations. MRS signals are weaker than those used by MRI, and the proper acquisition of the spectrum generally requires relatively large voxel sizes (poor spatial resolution) and long acquisition times (poor temporal resolution). Also, several compounds are not MRS visible, even when containing MRS-visible nuclei, owing to intrinsic molecular dynamics. Nonetheless, the ability to provide unique neurochemical information not accessible through other brain-imaging methodologies makes MRS an important tool in psychiatric research, and it is expected that future technical improvements will likely overcome some of the current shortcomings.

Another technique that makes use of the phenomenon of NMR to obtain neurochemical information is the magnetization transfer imaging (MTI). As MRS, this novel MR methodology utilizes the same magnets used to obtain structural MR images. While MRI explores the resonance of ^{1}H in free water to produce brain images, MTI identifies the resonance of protons that are bound to macromolecular structures, such as myelin, and therefore less mobile. The integrity of these macromolecular structures can be assessed through the exchange of magnetization between bounded protons and free water, denominated magnetization transfer ratio (MTR). MTR appears to be a sensitive methodology to identify subtle white-matter abnormalities that do not involve gross loss of volume, or an obvious focal lesion, and therefore are not detected on structural MRI [20]. Reductions in MTR have been reported in neurological conditions that involve white-matter lesions such as multiple sclerosis, and more recently this technique has also been applied to investigations in schizophrenia [21].

IV. FUNCTIONAL NEUROIMAGING

While structural and chemical brain imaging methodologies have remarkably bolstered our knowledge on the pathophysiological process involved in neuropsychiatric disorders, only functional neuroimaging can fully explore the temporal dynamics and regional neural activation underlying specific cognitive functions. Functional neuroimaging is at the forefront of scientific efforts to understand the mental phenomenon. Currently, this is the best method to evaluate the relationship between brain activation and a vast range of mental processes, from problem solving to consciousness. Based on the physiological rationale underlying the methods, we can divide the functional imaging technologies in two groups: those that evaluate an indirect measure of brain activation, such as regional blood flow and energy consumption, and those that directly assess the electrical and magnetical components of neural activity.

The connection between brain functioning and regional increases in blood flow was first proposed in the 19th century, but the scientific attention to this phenomenon had waxed and waned up to the 1950s, when the first measurements of regional blood flow with a diffusible radioactive tracer were made in animals (for a historical perspective of functional brain imaging, see Raichle [22]). Although the relationship of blood flow and neuronal activation is not fully understood, it is postulated that synaptic firing of a group of neurons results in a transient increase in the energetic demands of these cells, and in the local production of some metabolites that, eventually, will lead to local blood vessel response and consequent increased flow [23]. The spatial correlation between neural activation and hemodynamic changes is relatively precise, but the hemodynamic response is somewhat sluggish compared to the actual neuronal firing: blood flow begins to increase $\sim$2 seconds after neuronal activation, reaching its peak $\sim$5–7 sec [24]. Nonetheless, this robust physiological relationship was successfully explored initially through PET and SPECT methods, and more recently with functional MRI (fMRI).

Regional cerebral blood flow (rCBF) can be accurately and quickly measured with PET. Several radiotracers are available for these measurements, and $H_2^{15}O$ is the most widely used owing to, among other reasons, its short half-life (123 sec), which allows multiple repeat measurements in the same subject [15]. On the other hand, SPECT tracers only allow steady-state assessment of rCBF due to longer half-lives, with a relatively poorer spatial resolution when compared to PET [25]. However, the high costs involved in these procedures (particularly with PET) and the use of radioactive tracers represent relevant limitations to

their use. These shortcomings are helping to establish fMRI as the favored technique for rCBF measurements. fMRI is based on the fact that the hemodynamic response to neural activation usually surpasses the local oxygen needs, resulting in higher amounts of oxygenated and lower amounts of deoxygenated hemoglobin when compared with other surrounding areas. Since deoxygenated hemoglobin has more distinct magnetic properties than oxygenated hemoglobin, it may be unambiguously identified through NMR. Thus, neural activation leads to an increased rCBF, which results in a decrease in the content of deoxyhemoglobin in this specific brain region that can be quickly measured by the MRI scanner. This effect is termed BOLD (blood oxygen level dependent), and represents the most common fMRI approach to study brain activation [26]. Like PET, fMRI has the ability to map brain activation in the order of a few seconds, but with the advantages of having better spatial resolution, and being less expensive, noninvasive, and safer (no radiation involved).

fMRI has extended the work initiated with PET, due to its unique qualities, and has now a pivotal role in the functional mapping of the brain. Currently, the most important framework for the design of functional studies and interpretation of data is derived from cognitive neuroscience. In particular, the most widely used strategy consists of dissecting a simple cognitive task to its basic subunits, and to subtract the pattern of brain activation observed during the task of interest from a control task. For instance, one can subtract the map of activation after seeing a happy face from the activation after seeing a neutral face, in order to exclude brain activation related to the visual system, representation of human faces, etc., thus obtaining the activation correlated only with the identification of the emotion of happiness [27]. This methodology permits one to outline, either spatially and/or temporally, neural circuits that are active during specific cognitive processes. Cognitive subtraction is a powerful tool to examine the living brain, but is subject to some criticism, and more integrative and connectionist methodological approaches are now being utilized to address aspects of the mental phenomena that theoretically present less functional segregation [28]. A large number of studies have reported abnormal rCBF at rest or task-related for a variety of psychiatric syndromes and symptoms. An examination of all these findings is beyond the scope of this chapter; reviews of rCBF studies in mental disorders can be found elsewhere [12,29].

Increase in regional blood flow to the brain is not the only indirect physiological measurement of neuronal activation. Increased consumption of glucose is usually bound to increased rCBF in discrete neuronal areas. In fact, the first studies with PET utilized labeled glucose (^{18}F-2-fluoro-2-deoxy-D-glucose, or FDG) to map neural activation. It soon became clear, however, that the long half-life of FDG (110 min) would not allow quick and repeated measurements of discrete cognitive tasks. Nonetheless, interesting task-related regional changes in brain glucose metabolism could be identified with PET studies [30]. The increased blood flow in active brain areas is also correlated with localized tiny increases in temperature. A noninvasive methodology to record thermal information of cortical areas (thermoencephaloscopy; TES) is under investigation, and can potentially be a useful tool for functional neuroimaging [31].

Nonetheless, all methodologies assessing secondary physiological responses to neural activation, such as blood flow or glucose consumption, experience a critical weakness: these methods are not able to unambiguously track the time course of neural events. Neuronal activation occurs within milliseconds, while secondary increases in blood flow and energy consumption happen within a few seconds. Only direct recordings of the electric currents and magnetic fields that accompany the synaptic firing can provide such precise temporal resolution. Neuroelectrical brain measurements are based on the fact that synchronous activation of anatomically localized neurons results in electrical current strong enough to be detected at the surface of the head. Noninvasive recording of this effect is done by placing electrodes on the scalp surface; the more electrodes, the better spatial resolution. In essence, two basic types of neuroelectrical recordings can be performed: electroencephalography (EEG), comprising the examination of brain spontaneous electrical activity, and event-related potentials (ERP), which involves techniques to extract the characteristics of electrical events that are time-related to specific sensory, motor, or cognitive events [32]. ERP studies represent an essential methodology to evaluate functional integrity of sensory systems; moreover, this methodology allowed the identification of electrical potentials that are not directly related to sensory stimuli. These "endogenous" ERPs, such as P300 [33], have played a critical role in the examination of temporal and anatomical sequencing of neural activation related to cognitive processes such as attention, stimuli perception, and memory [34,35].

However, EEG and ERP can not be considered imaging methods in the same way that PET and

MRI. Although exhibiting superb temporal resolution, traditional electrophysiological methods provide poor spatial detail, which limits substantially the anatomical localization of neural activation. The distortion of the electrical signal caused by surrounding brain tissue, skull, and scalp is one of the reasons that account for the lack of detailed anatomical information of EEG/ERPs. On the other hand, the recording of the tiny magnetic fields that result from neuronal electrical activity can potentially provide better spatial resolution, since magnetic fields are not affected by the tissues and fluids they need to cross over.

This technique, named magnetoencephalography (MEG), requires more specialized facilities than EEG, such as a magnetically shielded room, and superconducting technology to record the in vivo brain magnetic fields, but offers similar temporal resolution with improved spatial definition. However, even with MEG, the anatomical resolution of electromagnetical technologies is strikingly inferior to PET or fMRI. Also, electrical or magnetic activities from structures below the surface of the cerebral cortex are difficult to register. Recent methodological strategies have been developed to combine the outstanding anatomical resolution of MRI with the unrivaled temporal definition of EEG/MEG. Brain electrical activity mapping (BEAM), quantitative EEG, and high-density electrical mapping (involving even 256 electrodes) are some of the methodologies utilized to provide a topographical analysis of the EEG/ERP signal. Multichannel MEG and magnetic-evoked field (the magnetic representation of the ERPs) studies have focused in source localization of neural activity by deriving coordinate transformations that permit one to locate magnetic fields in three-dimensional MR images; this technique is called magnetic source imaging (MSI).

These combined functional imaging techniques have been utilized to establish presurgical functional maps for the treatment of pathologies such as brain neoplasms and epilepsy, and more recently have been applied to the characterization of information processing in psychiatric disorders [36,37].

A novel and promising functional technique that may potentially provide insight on both hemodynamic and electrophysiological components of neural response is known as optical imaging. Initially utilized only on exposed living brain cortex [38], optical imaging has evolved into a noninvasive technique capable of mapping brain activity in vivo by way of measuring changes in the properties of light as it crosses different brain tissues. A basic assumption in this approach is that neuronal firing leads to rapid changes in the optical characteristics of the brain region under activation [39]. Malonek and Grinvald have shown that localized changes in light *scattering* have a strong temporal association with cortical activation [40], whereas localized changes in light *absorption* appear to follow a temporal pattern strikingly similar to the slow hemodynamic response of neural activity [41]. Although the physiological mechanisms underlying these phenomena are not completely clear, it is believed that hemoglobin is the major factor responsible for photon absorption [42], while changes in light scattering may be directly related with alterations on the neural membrane potential [43].

Near-infrared spectroscopy (NIRS) is currently the most common form of noninvasive functional optical imaging [44], employing light of long wavelength that characteristically crosses farther through brain tissue than visible light. Investigations combining NIRS with fMRI, PET, and evoked-potential methodologies [39,45] have been validating NIRS and demonstrating its ability to evaluate in vivo changes in rCBF and neuronal activation in humans. Although the spatial resolution of NIRS is still limited [44], it is expected that technical advances will bring further improvements for this promising neuroimaging methodology.

V. DISCUSSION

The elucidation of brain mechanisms involved in formation of the human mind, and how it emerges from the activation of billions of neural cells, is one of the most interesting and challenging scientific endeavors of our day. Currently, in vivo neuroimaging methods have allowed unprecedented studies of the living human brain in health and disease. Available imaging methods can provide a vast range of information, such as detailed anatomical resolution, accurate studies of distribution and function of neurotransmitter systems and intracellular metabolites, and functional activation maps describing the temporal and spatial features of information processing in the brain in virtually real time.

Nonetheless, significant questions still remain to be addressed in the understanding of brain functioning. Conceptual theories on how the brain works are extremely important in the interpretation of neurobiological data. Substantial evidence of functional segregation of several brain functions has been provided from lesion deficit studies, and the identification of "hot spots"—i.e., brain regions unambiguously activated during specific cognitive processes—seems to provide support to theoretical approaches that try to localize mental tasks

to discrete anatomical regions. However, the brain is a massively interconnected structure, with reciprocal communications linking practically every anatomical region. A connectionist view of human brain function proposes that several brain regions must be integrated to perform elaborate cognitive functions such as thinking and emotion. Although not excluding modular processing, an integrative framework may be more promising to raise the most critical hypotheses to be examined with neuroimaging studies in psychiatric disorders [28].

Another major challenge in designing neuroimaging studies is the precise definition of the mental processes being tested. Concepts such as emotion, attention, and perception are clearly broad and complex terms, based on psychological models that have been debated over the years. This point is of crucial interest for neuroimaging studies in mental illness, since most psychiatric syndromes present myriad symptoms that may be potentially related to distinct neuropathological processes. Also, the blurred boundaries between some psychiatric diagnoses represent another thorny issue when conceiving an experimental imaging approach. In this sense, brain imaging studies can play a formidable role by helping to dissect the neural processes involved in each component of cognitive processing. This knowledge may eventually allow the development of more precise definitions of mental functions and psychiatric symptoms. Moreover, neuroimaging can potentially provide very important contributions to the development of new models to explain the pathophysiology of mental disorders, and ultimately characterize, for instance, neurobiological markers of illness vulnerability or psychopathology severity. These developments could eventually result in more effective treatments for psychiatric illnesses.

It is essential to understand the weakness and strengths of imaging methodologies, though. Significant efforts are being directed to the improvement of the spatial and temporal resolution of these techniques, and also to be able to examine various neurotransmitter and intracellular signaling systems. Techniques that blend different methodologies, such as MEG + MRI (MSI), seem especially promising. Furthermore, the appreciation of pathological brain functioning will not be complete without a thorough investigation of the healthy brain. In this direction, the development of detailed brain atlases comprising different imaging modalities (anatomical, neurochemical, and functional) is crucial. However, most brain measurements are continuous instead of categorical, and there is an enormous interindividual variability, and consequent overlap of these measurements among psychiatric patients and healthy controls. To consider these issues, population-specific, diagnostic-specific, and developmental probabilistic atlases are in progress [46]. Moreover, the systematic brain mapping of nonhuman primates, and even other species, plays a vital role in setting the findings into an evolutionary perspective, providing a relevant theoretical framework for the identification of specialized neural systems in the human brain [47].

In summary, neuroimaging studies are helping to narrow the gap between clinical psychiatric manifestations and the underlying neuronal pathology. Numerous investigations have confirmed the presence of identifiable brain pathology in mental illness, from gross anatomical abnormalities to dysfunctional task-induced activation of cortical areas. Although no pathognomonic lesions have been identified so far, neuroimaging represents one of the most powerful and versatile methodologies for the study of the living human brain, and has started to provide significant advances in our knowledge of the neurobiology of mental illnesses.

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5

Classification of Childhood and Adolescent Psychiatric Disorders

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I. INTRODUCTION

In the past 20 years, the classification and diagnosis of psychiatric disorders in children and adolescents have undergone substantial change. For example, though long disputed, we now know that depressive disorders can and do occur in youths, as well as in adults [e.g., 1–3]. In general, we use classification systems to reduce complexity, to create order, and, in psychiatry and psychology, to inform treatment. They are heuristic systems that assume general similarities across individuals in particular groups (e.g., depressed vs. not; anxious vs. not) who show similar symptoms. For a psychiatric classification system to be optimally useful, it must reliably differentiate between groups based on established criteria, serve a practical function, predict future behavior, and adequately capture the construct it was intended to. In short, it must be reliable and valid. The Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) [4] is the most commonly used diagnostic system in the United States. It utilizes a categorical (as opposed to dimensional) classification system organized by symptom clusters and grounded in empirical findings. In this paper, the DSM-IV disorders will be reviewed as they relate to children and adolescents. First, we will discuss disorders typically diagnosed in infancy and childhood. Second, we will review syndromes that are generally diagnosed in adulthood and how they manifest in children. Specifically, for each diagnostic category, we will review information related to the definition and prevalence of the disorder, comorbidity with other psychiatric disorders, course and developmental considerations relevant to the disorder.

II. DISORDERS TYPICALLY DIAGNOSED IN CHILDHOOD

A. Disruptive Behavior Disorders

In DSM-IIIR [5], attention, conduct, and oppositional behaviors were newly grouped together under the rubric of disruptive behavior disorders. This diagnostic reorganization was partly due to accumulating evidence that the separation of attention and behavior problems was not empirically supported (e.g., 6). DSM-IV maintained this overall organization, but made some major changes to the classification of these disorders: (1) the creation of an overarching category for patients who experience difficulties with restlessness, impulsivity, and inattention: attention deficit hyperactivity disorder (ADHD) with three resultant subtypes; and (2) the creation of two subtypes for conduct disorder based on age of onset.

1. *Attention Deficit Hyperactivity Disorder (ADHD)*

ADHD is characterized by a persistent and developmentally inappropriate pattern of inattention and/or hyperactivity-impulsivity that is present before age 7 and causes functional impairment in at least two settings (e.g., school and home). Symptoms must be present for at least 6 months. In the DSM-IV, there are three ADHD subtypes: predominantly hyperactive-impulsive, predominantly inattentive, and combined type. Diagnosis of the subtypes should be based on the predominant symptoms for the last 6 months. These subtypes were developed empirically from the DSM-IV field trials, but little is known regarding how valid and valuable they are clinically. In support of their usefulness, in the field trials the subtypes were shown to have differing clinical pictures: inattentive patients were more likely to be female, and were older than the combined types. Combined-type youths were older than the hyperactive impulsive patients and showed greater clinical impairment than the other two types.

The prevalence of ADHD is estimated to be 3–5% in school-age children [4,7]. With the new DSM-IV criteria, preliminary studies suggest that rates have increased owing to the definition of the three subtypes. While much is unknown about the etiology of ADHD, there is now a fair amount of evidence from twin and family-genetic studies that suggest that this disorder runs in families [e.g., 8,9].

Comorbidity is very common among youths with ADHD, most commonly with conduct disorder [10,11,12] and oppositional defiant disorder [13]. Conduct disorder is characterized by serious and pervasive aggressive and antisocial behavior. The overlap between ADHD and conduct disorder has been found consistently in studies of children with both disorders and estimates suggest that 40%–60% of teens with ADHD meet criteria for conduct disorder. The prognosis is worse for those youths who have both ADHD and conduct disorder; these youths are at increased risk for substance abuse, school failure, and future occupational failure.

In regard to the course of the disorder, ADHD is most typically diagnosed in children and adolescents, and symptoms usually decrease in later adolescent and adult years. Although ADHD severity decreases on average with age, symptoms are persistent for many children; in one study, 4 years after initial diagnosis 80% of youths continued to meet criteria for ADHD [14]. Although ADHD is usually diagnosed in childhood, the disorder can be diagnosed in adults as long as symptoms were present before the age of 7. The concept of adult ADHD is not without controversy, however; some claim that adult ADHD is very rare [e.g., 15], while a growing body of evidence suggests that ADHD often persist in adulthood [16]. Some data that support the validity of ADHD in adulthood come from studies that show the children of ADHD adults to have increased prevalence of the disorder [17]. Other support comes from studies showing parents of youths with "persistent" ADHD being much more likely to have ADHD than parents of youths with remitted ADHD [18]. As noted in DSM-IV [4], ADHD's symptom pattern changes with development, often making diagnosis more difficult in adults. For example, disorganization and inattention rather than hyperactivity, are often prominent in adults. Predictors of a good outcome for adulthood include: mild initial severity of ADHD, a supportive family environment, higher intelligence, and a lack of comorbid conduct disorder.

2. *Oppositional Defiant Disorder (ODD) and Conduct Disorder (CD)*

ODD and CD are childhood disorders characterized by a stable pattern of defiant and/or aggressive behavior that causes functional impairment at home, at school, or in both settings. The diagnosis of CD requires that at least three characteristic behaviors (e.g., stealing, threatening, fighting, cruelty to animals or people) be present in the last 12 months, with at least one symptom present in the last 6 months. Children with this disorder often violate the rights of others, break serious rules, and are destructive and deceitful. DSM-IV criteria specify two subtypes of CD: childhood onset and adolescent onset.

Children with ODD are characteristically negativistic, defiant, and hostile toward authority figures. For a diagnosis of ODD, four defiant behaviors (e.g., temper outbursts, talking back, breaking rules) must be present frequently over a period of 6 months. There are no identified subtypes for ODD.

Prevalence rates for CD appear to be on the rise in the recent decades and may be higher in urban than in rural settings [4]. However, prevalence rates differ dramatically depending on the sample being studied and the assessment method used: for males younger than 18, rates range from 6% to 16%; for females, rates range from 2% to 9%. Similarly, depending on the sample and assessment method, rates of ODD from 2% to 16% have been found [4]. Rates for both behavior disorders are higher in boys than in

girls, though after puberty, rates for ODD become roughly equal.

As discussed above, comorbidity between CD and ADHD is quite common. CD and ODD are also commonly comorbid. Indeed, there is significant diagnostic overlap among the disruptive behavior disorders (particularly among very young children) as well as genuine cooccurrence of these conditions [19,20]. It has been suggested by some that hyperactivity is a necessary part of CD [21]. Thus, to appropriately guide treatment, thorough, multimethod, multi-informant evaluations are necessary to attempt to determine which children meet criteria for CD, ODD, and/or ADHD.

In terms of the course of these disorders, there is a good amount of support for the continuity between externalizing problems in preschoolers and CD in children who are school aged and older [e.g., 19,22]. In a review of longitudinal studies examining preschoolers with behavior problems, Campbell [23] showed that at least 50% with moderate to severe behavior problems continued to manifest such problems when they were school aged. Similarly, Richman, et al. [24] showed that almost 70% of preschoolers with disruptive behavior problems continued to have aggression problems when assessed 5 years later.

However, studies of young adults suggest that CD symptoms decline with age; in a longitudinal study of children with ADHD, Manuzza et al. [25] reported that the prevalence of CD dropped from 25% at age 18 to 15% at age 25. In a similar study of youths (6–17 years) with ADHD, Biederman et al. [14] found that CD symptoms persisted in only 42% of those originally diagnosed with subthreshold or full CD. Consistent with these findings are those of CD samples documenting similar decreases in CD symptoms over time [e.g., 26,27]. It may be that significant conduct problems still remain for most, but that previously obvious and overt behaviors become more covert with age and thus are less readily discerned using standard assessment procedures. When looking at the course of ODD, symptoms appear to be stable over time [28]. Importantly, ODD does appear to be validly distinct from CD: the developmental profile, sex distribution, and factor analytically derived behavioral dimensions differ between the two disorders [29–31].

B. Mental Retardation

Many definitions of mental retardation (MR) have been utilized over the years, differing primarily in emphasis, rather than specific content. According to the DSM-IV, MR is defined by intellectual functioning that is significantly below average (IQ of 70 or below) existing prior to age 18, with associated deficits in adaptive functioning. This definition incorporates all of the elements of the widely accepted description of MR developed by the American Association of Mental Retardation (AAMR). In the DSM-IV, MR is further classified according to severity: mild (IQ of 50–55 to 70), moderate (IQ of 35–40 to 50–55) severe (IQ of 20–25 to 35–40), and profound (IQ below 20–25). The AAMR recently abandoned such severity classifications in favor of classification based on the specific needs of individuals with MR. Despite such classification differences, it is widely accepted that the core features of MR are low intelligence and deficits in developmentally appropriate life skills.

Mental retardation occurs in $\sim 1\%$ of children and adolescents. However, prevalence estimates vary significantly depending on how mental retardation is defined, the sample selected, and the assessment tools used. Early research suggests that MR is more common with increasing age [32], in males [33], and in minority groups [34], though this last finding may be related to bias in assessment tools. Additionally, research indicates that the prevalence of MR decreases with age as functional impairment due to low cognition reduces with age [4]. Recent reviews of the literature related to the prevalence of MR have suggested that large gaps remain in our knowledge and have called for standardization of MR definitions and research methodologies [e.g., 35].

Comorbidity of psychiatric disorders among individuals with MR is significantly more common than in the general population [4]. For example, a recent study of > 6000 children identified 1.5% as having some sort of intellectual deficiency; of these, 32% were also identified as having a comorbid psychiatric disturbance [36]. The rates of comorbidity were significantly higher among those with intellectual deficiencies than among those who were not intellectually disabled [32% vs. 13.5%). Similarly, in an investigation of all children identified with MR in a Norwegian county, 37% were diagnosed with a comorbid psychiatric disorder, most commonly a pervasive developmental disorder [37]. Rates obtained were higher for those with severe MR than with those with mild MR: 42% and 33%, respectively. This pattern is consistent with the adult literature where those with more severe MR have significantly higher rates of comorbidity as well [e.g., 38].

The course of MR varies somewhat depending on the severity of the disorder, associated medical conditions, and environmental opportunities. As mentioned above, a diagnosis of MR necessitates that the disorder

be present prior to 18 years of age. More severe retardation tends to be diagnosed at younger ages, especially when associated with a characteristic, syndromal presentation (e.g., trisomy 21) [4]. MR is only diagnosed when clear deficits in adaptive behavior, judged within a developmental context, are present (assessed using standardized tools such as the Vineland Adaptive Behavior Scales). Such deficits can include impaired self-help skills, communication, academics, safety, and work performance. Less severe cases are often not diagnosed until children are old enough to have noticeable difficulties in school. Academic deficits are among the most easily documented and measured, perhaps accounting for the relatively high prevalence of MR during the school years [39]. Indeed, in adulthood, many individuals previously diagnosed with MR may be able to function adaptively enough outside of the academic arena that they are no longer classifiable as MR. That is to say, MR is not necessarily a lifelong disorder; for those adults who can develop good adaptive life skills in various domains (e.g., self-care and work), their level of functioning precludes an MR diagnosis [4].

C. Learning, Communication, and Motor Disorders

This group of disorders is characterized by academic, motor, or communication skills that are below developmental and intellectual expectations. Learning disorders are a fairly heterogeneous group of difficulties distinguished by academic achievement that is substantially below that expected for one's age, intellect, and/or schooling [4]. As a group they include: reading disorder, mathematics disorder, disorder of written expression, and learning disorder not otherwise specified. In the DSM-IV [4], it is specified that achievement deficits be measured by a standardized, individualized test and that they significantly interfere with academic performance or daily life.

Developmental coordination disorder, the only motor skills disorder, is characterized by a significant impairment in motor coordination that causes functional impairment; the specific manifestations of this disorder vary with age and development. The communication disorders include expressive language disorder, mixed expressive-receptive language disorder, phonological disorder, stuttering, and communication disorder not otherwise specified (NOS). Those diagnosed with expressive language disorder have deficient expressive language skills, including small vocabularies, few multiple-word combinations, and idiosyncratic word ordering. Mixed expressive-receptive disorder is characterized by delays in both expressive language and receptive language (i.e., comprehension). Phonological disorder is defined as the failure to develop typical speech sounds (e.g., ch, bu) at the expected age, and stuttering is characterized by speech dysfluency, syllable and sound repetition, and disrupted speech timing.

The prevalence of these disorders is thought to be relatively high, but estimates vary according to sample characteristics and measures used. According to most estimates, 5–15% of school-age children have learning disabilities [40], and these are diagnosed more commonly in boys than in girls [41]. Approximately 6% of young children have developmental coordination disorder [42], and 5–10% of children are estimated to have communication disorders [4,43]. Among the communications disorders, expressive language delays have been found to be the most common. Stuttering, in particular, is much more common in boys than in girls [3:1].

Many children with learning disorders also have associated comorbidities. Conversely, 10–15% of those with conduct disorder, oppositional defiant disorder, ADHD, and depressive disorder also have learning disorders [4]. Although developmental coordination disorder has not been well researched yet, associated difficulties are thought to include other developmental delays, in particular language delays [4]. More research has focused on communication disorders, and at this point, it is fairly well established that young children with communication difficulties are at increased risk for continued language problems, learning disorders, and psychiatric difficulties [e.g., 44–46].

Learning disorders as a group are thought to have a similar course over time. They are most commonly diagnosed in the elementary school years when academic challenges begin to go unmet. The school dropout rate for children with learning disabilities is 40%, significantly higher than the rate for those without such disorders [4]. Higher IQ is associated with better outcome for those with learning disorders. Depending on severity, learning disabilities may persist until adulthood and cause impaired occupational functioning [47].

Among the communication disorders, the course is more variable. Age of identification is predictive of language disorder severity, with later-identified children typically having more severe and persistent

delays. Phonological difficulties that are not severe are the most likely to resolve. Among those with expressive language difficulties, ~ 50% will "recover" and the rest will continue to manifest significant language difficulties [e.g., 48–50]. We know very little about the course of developmental coordination disorder; future research should examine the longitudinal course of significant motor skills deficits.

D. Pervasive Developmental Disorders

The pervasive developmental disorders (PDDs) are characterized by severe impairment in several crucial areas of development: communication, social interaction skills, and/or the presence of stereotyped behavior, play, or interests. These disorders are often diagnosed in the first years of life and are typified by behaviors and skills that are grossly developmentally delayed and/or inappropriate. PDDs as defined by the DSM-IV include autistic disorder (autism), Rett's disorder, childhood disintegrative disorder, Asperger's disorder, and pervasive developmental disorder not otherwise specified (PDD NOS) [4].

Autism's essential features include abnormal or impaired social interaction and communication and a severely restricted inventory of interests and activities. Delays or abnormalities must be present before the age of 3 in at least one of the following areas: social interaction, language in social communications, or symbolic/imaginative play. Most children with autism are also mentally retarded [4]. Rett's disorder, which has only been seen in girls, is distinguished by a period of typical functioning followed by the development of multiple specific deficits. For these children, head growth decelerates between 5 and 48 months, previously acquired hands skills are lost between 5 and 30 months, and subsequently, stereotyped hand movements similar to hand-wringing or hand-washing appear. In addition, interest in social interaction diminishes, expressive and receptive language are impaired, psychomotor retardation develops, and poorly coordinated gait or trunk movements appear. Childhood disintegrative disorder (CDD) is similar to Rett's disorder in that severe regression occurs after a period of typical development, but normal development must have lasted at least 2 years. A diagnosis of CDD requires that after the age of 2 (but before 10) there is a clinically significant loss of previously acquired skills in at least two of the following areas: expressive and receptive language, social skills or adaptive behavior, bowel or bladder control, and/or play or motor skills. Children with CDD exhibit social and communication deficits that are similar to those observed in children with autism. Asperger's disorder like autism, is characterized by persistent and severe impairments in social interaction and restricted, repetitive behavior, interests, and activities. However, with Asperger's disorder there are no characteristic language delays or deficits, nor are there cognitive impairments or deficits in age-appropriate adaptive behavior. PDD NOS is a diagnosis for those children who exhibit many, but not all, of the specific features required for a diagnosis of a specific PDD.

In terms of prevalence, PDDs are quite rare. Epidemiological studies suggest a rates of autism to be two to five cases per 10,000 individuals [4]. Data specific to prevalence for the other PDDs are very limited. Rett's disorder has only been discussed in limited case studies and only seen in females. Childhood disintegrative disorder (CDD) is thought to be very rare (much less common than autism) and more common in males than females. Asperger's disorder is also thought to be very rare, and also appears to be more common in males than in females.

PDDs are typically lifelong disorders with characteristic developmental shifts in symptom patterns. However, with early, intensive behavioral treatment some children with autism, Asperger's, or PDD NOS may benefit significantly enough that they lose their PDD diagnosis [51]. Unfortunately, this is not the case for most children; this sort of treatment is not widely available, and is variable in its success depending on factors such as severity of initial symptoms, comorbid mental retardation, and other individual differences that we do not yet well understand. Children with autism typically develop better functioning with age and often show some improvements in language and social interaction. For children with Rett's disorder there are characteristic developmental changes: between 1 and 3 years symptoms are very similar to those of autism, between 2 and 10 years of age, social interest increases somewhat, and after age 10, there are worsening motor problems [52]. For children with CDD, the long-term outcome is typically not very good, with little improvement in specific skills over time. Across the PDDs the prognosis is typically best for those with Asperger's, as they have communicative and cognitive skills that enable them to function well despite substantial social skill deficits. In adulthood, Asperger's (or mild autism) might be confused with schizoid or schizotypal personality disorders because of the overlapping social deficits [4].

III. PSYCHIATRIC DISORDERS USUALLY DIAGNOSED IN ADULTHOOD

A. Mood Disorders

1. *Major Depressive Disorder (MDD) and Dysthymia*

Although depression was not officially recognized as a disorder of childhood until 1980, at this point it is relatively well established that the clinical presentation of depressive disorders in children is similar to that seen in adults [4,53]. The DSM-IV uses adult criteria to diagnose depressive disorders in children, but places a greater emphasis on the developmental course of the disorder than DSM-III or DSM-III-R. Documented developmental differences in the presentation of MDD include increased suicide attempts and impairment in functioning with age, and decreased somatic complaints, phobias, and behavioral problems, which occur more in childhood than adulthood [e.g., 54].

In the DSM-IV [4], a diagnosis of MDD is made in individuals who demonstrate at least one major depressive episode (MDE) without previous experience of a manic, mixed, or hypomanic episode. Two weeks of a depressed mood or the loss of interest or pleasure in nearly all activities characterizes an MDE. In children, irritability rather than sadness can be the predominant emotion. In addition to a mood disturbance, at least four other symptoms from the following list must be present: changes in sleep, appetite/weight, or psychomotor activity; reduced energy; feelings of worthlessness or guilt; difficulty thinking concentrating or making decisions; and thoughts of death or suicidal ideation, intent, or plan. To be considered symptoms of MDD, these difficulties must represent a clear change from previous functioning.

Dysthymia is a more chronic depressive disorder characterized by similar symptoms of a lessor severity and longer duration (at least 2 years). In the following sections, we will focus primarily on MDD, as the majority of research data pertains to this diagnosis rather than dysthymia.

Depressive disorders in youths are not rare: population studies estimate that between 0.04% and 2.5% of children and 0.04% and 8.3% of adolescents have MDD [54] and $\sim$3% have dysthymia. In a large-scale study of adolescent psychopathology, MDD had the highest lifetime prevalence rate (20%) of all disorders surveyed, and a point prevalence rate of 2.92 [55]. These findings are consistent with other studies of adolescent depression, and with lifetime rates of MDD found among adults [e.g., 56,57]. In childhood, rates of depression are similar for girls and boys; in adolescence, however, the female-to-male ratio jumps to 2:1, which is comparable with ratios found in adult depression [e.g., 3,58).

MDD in youths is often comorbid with other psychiatric disorders. Rates of comorbidity in youths are comparable with, or slightly higher than, rates seen among adults with depression [59]. Epidemiological studies have shown that 40–70% of depressed children and adolescents have a comorbid psychiatric disorder, and that approximately 20–50% have more than one comorbid condition [e.g., 60–62). Dysthymia cooccurs with depression in $\sim$ 30% of youths and adults [59]. Diagnoses that are most commonly comorbid with depression in youths include anxiety disorders (30–80%), disruptive behavior disorders (10–80%), and substance abuse (20–30%) [63]. Indeed, anxiety disorders so commonly co-occur with depression that some have argued that they are manifestation of the same, not distinct disorders [64]. Others have cogently argued that although anxiety and depression do share some overlapping symptoms, the absence of positive affect is characteristic of depression, not anxiety [see 65].

Depression in youths is disabling and chronic, though perhaps somewhat less so than among adults. In a large, randomly selected sample of high school students, those who were identified as depressed were likely to have moderate to severe depression (88.6%) and to be judged in need of treatment (93.2%) [55]. The average length of a depressive episode is 9 months in youths [66], while on average 12 months for adults. Relapse rates are disturbingly high for children and adolescents with depression; $\sim$ 70% will relapse with in 5 years [55,59). Moreover, follow-up studies of depressed youths indicate that 20–40% will go on to develop bipolar disorder within 5 years of the onset of their depression [e.g., 67,68).

2. *Bipolar Disorders*

Though not without controversy, in recent years it has become more recognized that children can manifest symptomatology that is consistent with a diagnosis of mania, or bipolar disorder (BPD). Indeed, several exhaustive reviews of the literature have supported the validity of this diagnosis in youths [69–71]. According to DSM-IV, the occurrence of one or more manic or mixed episodes determines BPD. A manic episode is characterized by a distinct period of an abnormally elevated, irritable, or expansive mood that lasts at least a week. In addition to this mood

disturbance, three symptoms from the following list must be present: inflated self-esteem or grandiosity, decreased need for sleep, pressured speech, increased activity or psychomotor agitation, distractibility, flight of ideas or racing thoughts, or involvement in pleasurable activities with a high potential for negative consequences (e.g., buying sprees or indiscriminant sexual encounters). A mixed episode is defined as a period of at least 1 week during which criteria for both a manic and depressed episode are met. Often there is also a history of depressive episodes in these individuals. As classified in DSM-IV, bipolar disorders include: bipolar I, bipolar II, cyclothymic disorder, and bipolar disorder not otherwise specified. These disorders are differentiated based on the duration of symptoms and presence or absence of a full-blown manic or mixed episode.

Empirical work suggests that while bipolar disorder is difficult to diagnose in children, in part because it differs from adult mania in presentation, prevalence rates are higher than previously thought in youths, particularly among inpatients. However, few well-done studies of the prevalence of BPD exist. In one recent study of > 250 consecutively referred preadolescent children, Wozniak and colleagues [72] found that a surprising 16% met diagnostic criteria (DSM-III-R) for mania. In light of such high rates of mania documented by some researchers in specialty mood clinics and low rates seen in epidemiological studies (lifetime prevalence rate of 0.58) [73], the ''real'' prevalence of BPD in youths and how to best diagnose the disorder is still a hotly debated topic [see, 74–76]. To resolve this debate, more well-designed research needs to be conducted in the area of diagnosis and prevalence of pediatric bipolarity.

Comorbidity with childhood mania is the rule rather than the exception. However, the exact nature of the relationship between childhood mania and other disorders, in particular, attention deficit hyperactivity disorder (ADHD), is still being debated [77]. Indeed, symptom overlap with ADHD (e.g., impulsivity, concentration problems) is one of the most challenging aspects of accurately assessing and diagnosing bipolar disorder in children. Studies have shown rates of ADHD ranging from 60% to up to 90% among children with mania [72,78,79]. Studies involving children with bipolar disorder have also documented a high degree of overlap with conduct disorder [72,80,81]. For example, Kovacs and Pollack [81] reported that among children with BPD, an astonishing 69% also had conduct disorder. A recent epidemiological study also documented high rates of cooccurrence between these disorders [73]. Such complicating comorbidities, as is typical with other disorders, predict a worse course for these youths [e.g., 81].

Age of onset for a first manic episode is typically during late adolescence, but, as alluded to above, some cases start in early adolescence or childhood [4]. The course and presentation of pediatric mania is often atypical when compared to adult mania. Adult and adolescent mania is typically episodic with an acute onset, and is characterized by the presence of euphoric mood. With children, on the other hand, some have asserted that mania during childhood is characterized by a chronic, mixed mood state [72,76] and that the mood disturbance often manifests as irritability rather than euphoria [82,83]. In a recent review of the empirical literature related to pediatric bipolar disorder, it was concluded that, ''pre-pubertal BPD is a non-episodic, chronic, rapid cycling, mixed manic state.'' [70]. This suggests that BPD in youths is indeed atypical when compared to adult BPD, but that it is predictably atypical.

B. Anxiety Disorders

Excessive fear, distress, and/or avoidance of particular situations or objects, thoughts/memories, or physical sensations characterize anxiety disorders. In the DSM-III-R, three anxiety disorders were listed in the child section: overanxious disorder (OAD), separation anxiety disorder, and avoidant anxiety disorder. In DSM-IV, questions regarding the validity of these diagnostic categories led to a reorganization so that only separation anxiety disorder currently remains in the childhood disorders section (in the category ''other disorders of childhood''). In addition, the criteria for social phobia and generalized anxiety disorder were modified so that they incorporated the symptoms of children who would have been previously diagnosed with avoidant or overanxious disorder. As such, according to DSM-IV, the general anxiety disorders include: panic disorder with and without agoraphobia, specific phobia, social phobia, obsessive-compulsive disorder (OCD), posttraumatic stress disorder (PTSD), acute stress disorder (ASD), generalized anxiety disorder (GAD), anxiety disorder due to a general medical condition, substance-induced anxiety disorder, and anxiety disorder NOS. After describing each disorder, we will focus on those that are most relevant to children and adolescents and for which the most research exists: social phobia (previously avoidant disorder in children), generalized anxiety disorder (formerly overanxious disorder in children), and

separation anxiety disorder. Although, as previously mentioned, separation anxiety disorder is not formally classified with the anxiety disorders, we will discuss it in this section because of the considerable theoretical and clinical overlap it shares with the anxiety disorders as listed in DSM-IV.

In terms of the features that are specific to each disorder, panic disorder with and without agoraphobia is characterized by panic attacks (sudden onset of intense fear and physical symptoms such as racing heart, shortness of breath, feeling dizzy or faint) about which there is persistent concern. Avoidance of or anxiety about places from which escape would be difficult or embarrassing in the event of panic characterizes agoraphobia. Specific phobias are defined by significant anxiety related to a specific object (e.g., insects or needles) or situation (e.g., elevators or flying) which often leads to avoidance. Social phobia is characterized by anxiety and resultant avoidance related to social or performance situations. OCD is defined by the presence of intrusive, upsetting thoughts (obsessions) and compulsions (repetitive or ritualized behaviors or mental acts) designed to reduce anxiety. PTSD is characterized by reexperiencing (e.g., in nightmares or intrusive thoughts) a traumatic event accompanied by avoidance of trauma-related stimuli and increased arousal (e.g., sleep and concentration difficulties). Acute stress disorder is defined by symptoms that are similar to those of PTSD (with an emphasis on dissociative symptoms) that occur very soon after the traumatic event. GAD is typified by persistent worry and anxiety that is difficult to control that lasts at least 6 months. Separation anxiety disorder is characterized by developmentally inappropriate anxiety (lasting at least 4 weeks) regarding separation from the home or people to whom the child is attached.

Anxiety disorders are among the most commonly diagnosed psychiatric disorders in both children and adults [84]. Epidemiological studies show rates of anxiety disorders ranging from 5.7% to 17.7% in children and adolescents [e.g., 57,61,85,86]. Additionally, these studies show a trend for rates of anxiety disorders to increase with age. Looking at specific disorders, epidemiological studies show prevalence rates as follows: social phobia, 0.06%–7.9%; GAD/OAD, 2.9–10.8%; and separation anxiety disorder, 2.0–4.7%. Though there is a good deal of variability in these estimates, GAD appears to be most common in youths, followed by social phobia, which is also very common.

Anxiety disorders in children (specifically social phobia, GAD, and separation anxiety disorder) are often comorbid with other psychiatric disorders. Indeed, for most children with significant anxiety, comorbidity is the rule rather than the exception. As mentioned previously, depression and anxiety in particular very commonly cooccur; anxiety disorders are three to four times as likely to occur in youths with depressive disorders as in youths without such disorders [e.g., 85,86]. Anxiety disorders are also often comorbid with disruptive behavior disorders. Several studies have found them to be two to three times more common among children with ODD and CD [e.g., 85–87].

Data regarding the course of anxiety disorders in youths are scarce. In a recent study, children diagnosed with an anxiety disorder were followed up 3–4 years later [88]. Eighty percent of the children had recovered from the originally diagnosed disorder, and only a small percentage (8%) experienced a relapse of their disorder. However, these children were likely to develop new disorders. These results are consistent with findings from a 5-year follow-up study of children and adolescents initially diagnosed with anxiety disorders; at follow-up, most of the children had either recovered from the initial diagnosis or had developed a different disorder—most typically, a different anxiety disorder [28]. To date, two studies have found that continuity of anxiety disorders is more common among girls than among boys [85,89].

C. Psychotic Disorders

Psychotic disorders are characterized by the presence of hallucinations or delusions, and grossly disorganized behavior or speech. As with affective disorders, the assumption in DSM-IV is that adult criteria for psychotic disorders should be extended downward to apply to children. However, it has been suggested that the lack of specific attention to developmental issues and the focus in DSM-IV on disorganized speech may lead to errors of overdiagnosis in children [90,91]. In DSM-IV, the psychotic disorders include schizophrenia, schizophreniform disorder, schizoaffective disorder, delusional disorder, brief psychotic disorder, shared psychotic disorder, psychotic disorder due to a medical condition, substance-induced psychotic disorder, and psychotic disorder NOS.

The psychotic syndromes are differentiated based on duration and pattern of presenting symptoms. Schizophrenia is characterized by the presence of at least two of the following symptoms present for at least 1 month: hallucinations, delusions, disorganized speech or behavior, and negative symptoms (e.g., anhedonia, avolition). The disturbance must last at least 6

months overall and cause significant clinical impairment. In terms of symptoms, schizophreniform disorder is the same as schizophrenia, but does not last as long (1–6 months) and need not cause functional impairment. Schizoaffective disorder is typified by a mood disturbance that occurs simultaneously with the positive symptoms of schizophrenia, and is preceded by at least 2 weeks of delusions or hallucinations without concomitant mood disturbance. Delusional disorder is characterized by at least 1 month of delusions that are not bizarre in content. Shared psychotic disorder is a disturbance that develops in one person owing to the influence of another person with a similar delusion.

Psychotic disorders in children are considered rare, but few studies have been conducted in this area, and most focus exclusively on schizophrenia. The prevalence of schizophrenia in very young children (12 years old and younger) has been estimated at rates of 1.6–1.9 per 100,000 [92,93]. Among adolescents, rates of schizophrenia are estimated at 0.23% in the general population, 1% among outpatients [94], and 5% among inpatients [95]. Among adults, estimates of schizophrenia prevalence rates range from 0.2% to 2% [4]. Schizophrenia with onset at a very young age is about twice as likely in males as in females [96].

In youths with psychotic disorders it is thought that comorbidity is fairly common, particularly with disorders of behavior, attention, and motor skills. However, little empirical work exists in this area. Histories that are suggestive of premorbid pervasive developmental disorders are common [97,98], as are comorbid behavior and attention problems [99]. Results of one study indicated that among youths diagnosed with schizophrenia, $\sim$13% had a history suggesting preexisting attention or motor skills deficits [100].

Psychotic disorders are typically first diagnosed in the late teens through early 30s, with onset before the teen years being uncommon [4]. Age of onset has been found to be prognostic: children with very early onset schizophrenia tend to have a very poor prognosis [91,101]. In general, studies of adult schizophrenia show a variable course of the disorder, with some individuals remaining chronically ill, and others experiencing periods of remission and exacerbation [4]. There are some common developmental variations in symptoms: in children, visual hallucinations may be more common than in adults, and hallucinations/delusions may be less elaborate. Delusions are only seen in $\sim$50% of cases of childhood schizophrenia [102,103]. Additionally, disorganized speech is common to several disorders typically seen in children (e.g., pervasive developmental disorders, communication disorders), so this symptom is less indicative of a psychotic disorder in this group then when seen in adults. Acute onset of the disorder is more likely the older the age of the child [96].

IV. CONCLUSIONS

This chapter has reviewed many of the major diagnostic classifications as listed in DSM-IV and highlighted diagnostic issues that pertain to children and adolescents. Overall, psychiatric disorders are common in youths and typically increase in prevalence with age. Comorbidity is also quite common in children and adolescents, and is associated with poor outcome. The course of the various psychiatric disorders is variable, but on average, early age of identification predicts a more chronic course, and as such, may serve as a proxy for severity. In terms of developmental sensitivity, in the DSM-IV, there are very few diagnostic criteria differences across the life cycle, but the presentation of symptoms is modified and mediated by developmental influences. As such, we have attempted to outline characteristic developmental symptom patterns for each disorder.

As we noted at the start of this chapter, the classification and diagnosis of psychiatric disorders in children and adolescents have undergone substantial change and progress in the past 20 or so years. We, as a field, have begun to accumulate empirical work that to varying degrees support or make us question our diagnostic categories as they now stand. More research that examines the presentation, course, and outcome of various psychiatric disorders in youth is needed, particularly in the area of bipolar disorders, pervasive developmental disorders, and psychotic disorders.

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6

Classification of Schizophrenia and Related Psychotic Disorders

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I. INTRODUCTION

The need for a classification of mental disorders has been clear throughout the history of medicine, but there has been little agreement on which disorders should be included and the optimal method for their organization. The many nomenclatures that have been developed during the past two millennia have differed in their relative emphasis on phenomenology, etiology, and course as defining features. Some systems have included only a handful of diagnostic categories whereas others have included thousands. Moreover, the various systems for categorizing mental disorders have differed with respect to whether their principal objective was for use in clinical, research, or statistical settings [1].

Attitudes to psychiatric classification have also undergone a revolution in the last generation. In the 1950s and 1960s, psychiatric diagnoses did not occupy center stage in clinical practice. Their reliability was known to be low; it was known that key diagnostic terms like schizophrenia had different meanings in different parts of the world. On the other extreme, there were some who argued that diagnostic categories should be abandoned and they believed that all patients require the same treatment—the ''moral regime'' of the asylum for Neumann and Prichard in the 19th century, and psychotherapy of Rogers and Menninger in the 20th century [2].

However, a clear definition and accurate classification of a disorder are the first steps in any systematic attempt to understand the pathophysiology and etiology of the disorder. The revolution in biological psychiatry can, in part, be attributed to advances in nosology [3].

II. EVOLUTION OF CLASSIFICATION SYSTEMS

Ethnographic studies have demonstrated that schizophrenia is present in all existing cultures, from the preliterate to the most advanced. Psychotic symptomatology and schizophrenialike syndromes were clearly present in ancient civilizations. However, more accurate and systematized classifications of psychological disturbances began to evolve only in the 1st and 2nd centuries AD. The physician Aretaeus of Cappadocia defined a state of melancholy, which included depression as well as schizophrenialike withdrawal. In the 1700s there was an increasing emphasis on detailed and accurate descriptions of abnormal mental processes and states. Philippe Pinel, a French physician, considered to be one of the founders of modern psychiatry, argued for an objective medicophilosophical approach to psychological disorders. Jean Etienne Esquirol, a student of Pinel, defined hallucinations

and identified "monomania", a clinical syndrome similar to modern descriptions of paranoid schizophrenia.

Attempts were also being made to divide the clinical landscape into syndromes sharing both clinical features and course. Benedict Augustin Morel was the first to use the term dementia praecox (*dementia precoce*). Other symptom complexes identified included delusional states (France) and paranoid states, as described by the German physician Vogel in 1764. Johann Christian Augusts Heinroth outlined 48 distinct disease entities and thereby epitomized the general inability to develop straightforward, reliable criteria. These theoretical controversies and confusion led Heinrich Neumann to reject all systems of classifications and suggest that it was necessary to "throw overboard the whole business of classifications" to bring order to the field. He suggested that "there is but one type of mental disturbance, and we call it insanity." Nevertheless, despite the intermittent sense of frustration and confusion, classificatory efforts continued unabated [4].

III. 20TH CENTURY CLASSIFICATORY SCHEMAS OF KRAEPLIN AND BLEULER

It was in the latter part of the 19th century that Emil Kraeplin was able to integrate the diverse clinical phenomena into a coherent and far-reaching classificatory system. His synthetic formulation included the identification of "dementia praecox" to refer to the clinical entity we now call schizophrenia. "Dementia" referred to the progressive deteriorating course of both emotional and cognitive processes; "praecox" indicated the early age of onset in previously healthy individuals. Thus, fundamental to the diagnosis were both cross-sectional and longitudinal components. Importantly, he differentiated the generally deteriorating course of dementia praecox from the more episodic and customarily better outcome seen in manic-depressive disorder. He furthermore divided it into four subtypes: paranoid, hebephrenic, catatonic, and simple.

Eugen Bleuler used Kraeplin's systematic classification of psychoses and a theoretical model of etiological processes to reformulate dementia praecox as "schizophrenia," derived from the Greek words for "split" and "mind" [5]. He asserted that there were four cardinal features almost invariably present in schizophrenia patients, the "four A's": blunted affect, loosening of association, ambivalence, and autism. He viewed schizophrenia as being composed of several different entities rather than a single disease state as Kraeplin conceptualized. Other symptoms of schizophrenia include delusions, catatonia, negativism, and stupor. These were thought to be "secondary" symptoms and to present in reaction to the individual's intentions, drives, psychotic state, and environmental conditions. Bleuler noted that these secondary symptoms were present in schizophrenia as well as in other disorders. He also asserted that despite the secondary nature of these symptoms, they formed the basis of Kraeplin's classificatory system.

It is noteworthy that two psychotic features emphasised by today's Diagnostic and Statistical Manual (DSM)—hallucinations and delusions—were not crucial for Bleuler's diagnosis of schizophrenia. His emphasis on theory as a means for determining the diagnostic relevance of signs and symptoms contrasted sharply with Kraeplin's reliance on empirical observations. Bleuler's approach was also notable for three other reasons. First, his reformulation of dementia praecox as "the group of schizophrenia" foreshadowed the contemporary view that schizophrenia is a heterogeneous group of disorders with similar clinical presentations. Second, he included defects in affect as a core feature of the disorder. Third, his view of schizophrenia allowed for the possibility of recovery.

Other clinicians also advocated a hierarchical system of symptom classification like Bleuler. In 1959, Kurt Schneider termed the core features "first-rank symptoms". These symptoms included: hearing one's thoughts spoken aloud; auditory hallucinations commenting on one's behavior; thought withdrawal, insertion, and broadcasting; and somatic hallucinations, or the experience of one's thoughts as being controlled or influenced from the outside.

Manifestations of first-rank symptoms in the absence of organic disease, persistent affective disorder, or drug intoxication, were sufficient for a diagnosis of schizophrenia. Second-rank symptoms included other forms of hallucinations, depressive or euphoric mood changes, emotional blunting, perplexity, and sudden delusional ideas. When first-rank symptoms were absent, schizophrenia might still be diagnosed if a sufficient number of second-rank symptoms were present. Although the schneiderian criteria have been criticized as being nonspecific, they have been incorporated into clinical diagnostic tools such as the Research Diagnostic Criteria (RDC) and Diagnostic and Statistical Manual of Mental Disorders (DSM) classificatory systems [4].

IV. CLASSIFICATION ON THE BASIS OF SYMPTOMS

It is widely believed that classification of diseases should, wherever possible, be based on etiology. Unfortunately, the same principle does not apply to psychiatric disorders, since the etiology of most is still unknown or all that is known for certain is that both genetic and environmental factors are involved. For this reason, most contemporary classifications of psychiatric disorders are largely based on clinical symptoms. This state of affairs has a number of important consequences. Decisions about the presence or absence of symptoms are relatively unreliable; and because few psychiatric conditions have pathognomonic symptoms, most conditions have to be defined by the presence of some or most of a group of symptoms rather than the presence of one key symptom. In the jargon of nosology, they are polythetic rather than monothetic. This invites ambiguity and lowers reliability still further, unless operational definitions are adopted. Another important consequence is that most psychiatric diagnoses can never be confirmed or refuted, for there is no external criterion to appeal to.

For these and other reasons it has often been suggested that symptoms should be ignored and a new classification developed on an entirely different basis. Psychoanalysts have frequently advocated a classification based on psychodynamic defense mechanisms and stages of libidinal development. In the 1950s, clinical psychologists extolled the advantages of a classification based on scores on batteries of cognitive and projective tests. More recently, learning theorists have argued that we should classify patients on the basis of a comprehensive analysis of their total behavioral repertoire. In principle, all of these approaches are perfectly legitimate. In practice, however, none of them has ever progressed beyond the stage of advocacy. Two other alternatives proposed are (1) classification on the basis of treatment response, and (2) classification on the basis of the course or outcome of the illness. Unfortunately, neither is feasible since there are few if any specific treatments available in psychiatry, and most disorders can have a wide range of outcomes. It is sometimes assumed that Kraeplin's classification or at least his distinction between dementia praecox and manic-depressive insanity, was based on long term outcome, but this is a misunderstanding. Kraeplin certainly emphasized the difference in the lifetime course of his two great rubrics, and perhaps subdivided the functional psychosis in the way he did to maximize the difference in outcome between them. But he used outcome as a validating criterion (i.e., as evidence that his two rubrics were fundamentally different disorders), not as a defining characteristic. Thus, when patients with dementia praecox recovered completely, he would automatically have changed their diagnosis [2].

As things stand, we have no choice but to use a classification, which is largely based on symptoms, despite its shortcomings and imperfections, because no practical alternative has yet been developed. Kraeplin's and Bleuler's observations evolved into today's psychiatric classification: the International Classification of Diseases (ICD) and the American Psychiatric Association's Diagnostic and Statistical Manual (DSM) [5].

V. DIAGNOSTIC CRITERIA: DIAGNOSTIC AND STATISTICAL MANUAL OF MENTAL DISORDERS (DSM)

In this chapter we shall discuss the evolution of the different classification systems over time using the diagnosis of schizophrenia as an example. We will first address the reliability and validity of DSM and then address how ICD later synchronized with the DSM system.

A. DSM-I

In 1949, the American Psychiatric Association in collaboration with the New York Academy of Medicine began an initiative to standardize the diagnostic system throughout the United States. The result was the Diagnostic and Statistical Manual of Mental Disorders-1 (DSM-I), published in 1952. It was influenced by the theories of Adolf Meyer, and psychiatric disorders were viewed as reactions of the personality to psychological, social, and biological factors [4]. In addition to its use of Kraeplin's and Bleuler's views on the signs and symptoms of schizophrenia, the first DSM defined schizophrenia in a way that at least implied environmental causes. For example, all schizophrenic (and other psychiatric) diagnoses included the term "reaction" (as in "schizophrenic reaction, simple type"). Moreover, definitions were vague and did not discuss differential diagnosis. Such imprecise definitions allowed clinicians much discretion in making a diagnosis. As a result, in the United States, schizophrenia became the diagnosis of choice for psychotic conditions that lacked a clear "organic aetiology" [5].

B. DSM-II

The manual had gone through several major revisions. The DSM-II was published in 1968, but did not differ significantly from its predecessors [4]. It dropped the term "reaction" from its diagnoses and added some discussion of differential diagnoses, but continued the DSM-I tradition of brief, vague descriptions of schizophrenia disorders, without specific operational criteria. Interestingly, both of these early systems viewed psychosis as the key feature of the disorder. DSM-II did not contain a category ("schizophrenia latent type") to describe people with "clear symptoms of schizophrenia but no history of a psychotic schizophrenia episode." This category was intended to encompass individuals with a variety of conditions (e.g., "incipient," "prepsychotic," and "borderline schizophrenia," as well as "schizophrenic reaction, chronic undifferentiated type," from DSM-I). This did not reflect an important attempt to clarify the role of psychosis in schizophrenia illness [5]. Thus, the diagnosis, with schizophrenia as an example, lacked validity and was too vague in its description.

C. DSM-III

DSM-III was radically different from any previous classification. Published in 1980, it brought about a sea change in psychiatric classification, spearheaded by the "neo-Kraeplinian" movement in the 1960s and 1970s and by investigators in psychiatry and clinical psychology who emphasized the importance of empirical, psychometric validation of psychiatric syndromes. Its innovations were a response to the evidence that had accumulated over the previous 20 years that psychiatric diagnoses were generally unreliable, that there were systematic differences in the usage of key terms like "schizophrenia" between the United States and other parts of the world [2]. It contained several innovations, including field tests of diagnostic reliability, specific inclusion and exclusion criteria for diagnoses, multiaxial diagnosis, and a focus on the description of syndromes and course of disorders rather than inferences about their etiology. This last point made psychiatric diagnosis more explicitly consistent with the diagnosis of other medical disorders of unknown etiology.

The traditional distinction between neuroses and psychoses was abandoned to allow all affective disorders to be brought together. Also, in the absence of data to support diagnostic hierarchies, the system encourages comorbidity. DSM-III's use of clearly defined criteria limited the clinician's discretion and narrowed the construct of schizophrenia. This development improved the clinical homogeneity of the disorder, better delimited it from other serious mental illnesses, and raised diagnostic reliability to respectable levels. Nevertheless, DSM-III retained the view that psychosis was fundamental to the definition of schizophrenia. Fewer patients now had the diagnosis of schizophrenia, and more were diagnosed as having unipolar or bipolar affective disorder.

However, many senior American psychiatrists criticized this classification and its principal architect Robert Spitzer for introducing what they regarded as a crude "Chinese menu" approach to diagnoses, with a theoretical bias and phenomenology being favored over mental processes.

D. DSM-III-R

DSM-III was replaced by an extensive revision DSM-III-R (revised) in 1987. In this classification schizoaffective disorders were given an operational definition for the first time; the definition of paranoid disorders was enlarged to include patients with grandiose, somatic, and erotomanic delusions as well as with delusions of persecution and jealousy, and the inappropriate stipulation that schizophrenia must start before the age of 45 years was dropped. Being introduced only 7 years after DSM-III, this classification was criticized for disrupting research and practice because of the evolution of new definitions [2].

E. DSM-IV and DSM-IV-TR

The primacy of psychosis defining schizophrenia also survived DSM-III's revision and its evolution into DSM-IV (published in 1994) and DSM-TR (text revision published in 2000) [6]. DSM-IV was published in 1987 with the following goals [3]:

1. To develop criteria that are more constant with ICD-10, with regard to schizophrenia. Primarily, this had to do with changing the required duration of the psychotic symptoms from 1 week (as in DSM-III-R) to 1 month (as in ICD-9 and ICD-10)
2. To provide a simplified criterion of symptoms by reducing redundancy in the items of criterion A.
3. To include symptoms with proven reliability.
4. To include symptoms only with acceptable prevalence.
5. To provide maximum coverage (sensitivity) for existing cases, thus reducing the reclassification rate.

These goals were met by adopting a "thorough process" by the Psychotic Disorders Work Group, which consisted of comprehensive reviews of literature, reanalyses of previously collected data, input from the field, and issue-focused field trials that included testing of alternative sets of diagnostic criteria. Changes proposed ranged from minor modifications in the DSM-III-R criteria to more weightage for negative symptoms, expansion of the minimum duration of symptoms to 2 weeks or 4 weeks, to the introduction of a concept of "schizophrenia spectrum disorders."

Psychosis was deemphasized in DSM-IV, in that a patient could receive a diagnosis of schizophrenia according to DSM-IV criteria without having delusions or hallucinations. In that case, however, gross disorganization of speech and/or behavior, which are also psychotic symptoms, would still be required because criterion A (i.e., characteristic symptoms) requires at least two of the five symptoms in the category. Thus, four of the five symptoms are still related to psychosis (negative symptoms are the fifth symptom in the category). Moreover, delusions alone can satisfy the criterion if they are bizarre, and hallucinations alone can satisfy the criterion if they involve one or more voices engaging in running commentary or ongoing conversation. Diagnostic changes in DSM-IV thus expanded the nature of the required psychotic symptoms more than they deemphasized psychosis itself [5]. In the DSM-IV "Schizophrenia and other related disorders" include schizophrenia, delusional disorder, and schizoaffective disorder. Schizophrenia is divided into five subtypes including paranoid, disorganized, catatonic, undifferentiated, and residual [4]. The criteria for schizoaffective disorder has been changed to focus on an uninterrupted period of illness rather than on the lifetime pattern of symptoms. In Brief Psychotic Disorder, eliminating the requirement for a sever stressor has broadened the DSM-III-R construct of Brief Reactive Psychosis, and the minimum duration of the psychotic symptoms has been increased from a few hours to 1 day.

The importance of psychotic symptoms in diagnosis extends to other diagnostic systems. Schneider's first-rank symptoms, which form the basis of "nuclear schizophrenia," are types of hallucinations and delusions that have come (more than other, "second-rank" symptoms) to characterize the nature of psychosis in the disorder. More important, they have helped to define the disorder itself, although Schneider himself reviewed them more as diagnostic tools than as theoretical constructs about the etiology of the disorder. First-rank symptoms heavily influenced the development of Research Diagnostic Criteria for schizophrenia, which in turn formed the basis of DSM-III criteria for schizophrenia. These criteria, particularly, continue to influence ICD-10 in the first three symptom groups "that have special importance for the diagnosis" for schizophrenia [5].

VI. DIAGNOSTIC CRITERIA: INTERNATIONAL CLASSIFICATION OF DISEASES (ICD)

The Mental Disorders section of ICD-6 was primarily a classification of "psychoses and mental deficiency." The eighth revision of the International Classification of Diseases, Injuries and Causes of Death (ICD-8) came into use in 1969, owing to strenuous efforts by the World Health Organization. It was replaced by ICD-9 a decade later, in 1979. However, the definitions provided in ICD-8 and ICD-9 were not operational definitions [2].

A. Preparation of ICD-10

The process of drafting ICD-10 started in 1983 but it came into use in United Kingdom and most other countries in 1993. It had a new title, the International Statistical Classification of Diseases and Related Health Problems, and a new alphanumeric format. The main purpose of the latter is to provide more categories and so leave space for future expansion without the whole classification having to be changed. It incorporates many of the radical innovations introduced in DSM-III. Most categories are provided with both diagnostic guidelines for everyday clinical use and separate "diagnostic criteria for research," providing unambiguous rules of application. There is also provision for multiple axes, as in DSM-III and its predecessors.

Field trials of the 1986 draft text were held in 194 different centers in 55 different countries, and the final text benefited greatly from the comments of users in these varied settings and the evidence they provided of the acceptability, coverage, and interrater reliability of the provisional categories and definitions of the draft [2].

B. Differences between ICD-9 and ICD-10

F20–F29, which included schizophrenia, schizotypal states, and delusional disorders have been expanded by the introduction of new categories such as undiffer-

entiated schizophrenia, postschizophrenic depression, and schizotypal disorder. The classification of acute short-lived psychoses, which are commonly seen in most developing countries, is considerably expanded compared with that in the ICD-9 [7].

VII. OTHER PSYCHOTIC DISORDERS: DSM AND ICD DEFINITIONS

A. Schizoaffective Disorder

The study of Schizoaffective Disorder, since it has generally been ill defined, has always presented unique problems due to the lack of producing comparable populations. Several conceptual models of schizoaffective disorder exist, e.g., the episode-based versus the course-based co-occurrence of mood and psychotic symptoms. Both the ICD and the DSM use a common definition of episode-based coexistence of symptoms and are very similar in the other criteria. A rare, small subgroup of patients may, however, be diagnosed as Schizophrenia by the DSM-IV, and Schizoaffective Disorder by the ICD-10. This occurs because the ICD requires that at least 2 weeks of psychosis precede any concurrent psychotic and mood symptoms of schizophrenia, whereas the DSM does not. A patient presenting with concurrent psychotic and mood symptoms from the onset of the episode could be diagnosed Schizophrenia by DSM-IV if satisfying all the other criteria, but might be diagnosed Schizoaffective by ICD-10.

B. Delusional Disorder

For delusional disorder it is likely that some difference in subject selection will persist between the two major symptoms because of differing requirement in duration, i.e., 1 month in DSM versus 3 months in ICD.

C. Acute/Brief Psychotic Disorder

Although termed differently, acute psychotic disorders provide substantially the same coverage in the two systems. In DSM, the terms brief psychotic disorder and schizophreniform disorder are used, while the ICD uses the terms acute and transient disorder with and without schizophrenialike symptoms [3].

VIII. INTERNATIONAL DIFFERENCES IN DIAGNOSTIC CRITERIA

A. Diagnostic Hierarchies

In ICD-10, schizophrenia and affective disorders are at the same level. A diagnosis of schizophrenia cannot be made if the full depressive/manic syndrome is also present "unless it is clear" that schizophrenic symptoms antedated the affective disturbance.

However, in the DSM classification, schizophrenia traditionally follows the "organic psychosis," and the third place in the hierarchy is occupied by the affective disorders. A very similar sequence is involved in the decision pathway of computer programs like Catego [2].

B. Threshold for Diagnosis

Comparative studies carried out by the US/UK diagnostic project in 1960s established that, in comparable series of patients, psychiatrists in New York diagnosed schizophrenia twice as frequently as their counterparts in London. The International Pilot Study of Schizophrenia confirmed that American psychiatrists had an unusually broad concept of schizophrenia, and also showed that the same was true of Russian psychiatrists. The very broad American concept of schizophrenia was psychoanalytic in origin, and the decline of psychoanalytic influence in the 1970s, together with a renewed interest in descriptive psychopathology and classification, led to rapid change. The widespread adoption of the operational definitions of DSM-III and DSM-III-R by research workers in many different parts of the world has also played an important role in reducing the international differences in usage [2].

IX. RELIABILITY AND VALIDITY: THE PREREQUISITES OF A CLINICAL DIAGNOSES

The introduction of structured interviews and operational definitions has improved the reliability and validity of psychiatric diagnosis over the years. But the existing evidence for the validity of most psychiatric diagnoses is rather meager. It is considerably better for the major syndromes like schizophrenia in comparison to sub-categories of major syndromes such as catatonic schizophrenia [2].

A. Reliability and Field Trials

Modern classification schemes such as ICD-10 and DSM-IV have made it possible to assign psychiatric patients reliably to different diagnostic categories [8]. A classificatory system, which has little reliability, has little practical utility [9]. Field trials have been conducted at seven USA sites (each of which contributed 50 subjects) to assess the concordance and symptom reliability within different systems, namely, DSM-III, DSM-III-R, and ICD-10 [1]. Some of the major highlights of the results were:

1. Concordance between diagnostic systems.
2. Symptom reliability.
3. Reliability of diagnostic criteria.
4. Agreement between ICD and DSM-III-R was high (87.6%) for schizophrenia, but 13% of DSM-III-R schizophrenia was classified by ICD as schizoaffective, acute and transient psychotic disorder, schizotypal, or none of the above.
5. Reliability of schneiderian symptoms was similar to that of other symptoms. Likewise, bizarre delusions were as reliably rated as nonbizarre, and even negative symptoms had good reliability in the trial.
6. The length of symptoms is a principal difference in criteria between ICD and DSM. The field trial demonstrated that $\sim 5\%$ of DSM-III-R schizophrenia and $>30\%$ of schizophreniform disorder would have to be reclassified to psychosis not otherwise specified (NOS) when the required duration of symptoms is changed to 1 month.

B. Validity of Classification Systems

As yet no clinical or pathological gold standard exists for the diagnosis of schizophrenia. The uncertain validity of the diagnostic categories assigned to the patients is a matter of serious concern because the usefulness of a particular diagnostic construct is greatly reduced if it carries no therapeutic implications.

The validity of diagnostic classification rests to some extent on its ability to predict outcome. In a study by Mason et al. [10], it was found that DSM-III-R and ICD-10 diagnosis of schizophrenia had high predictive validity and were superior to ICD-9. ICD-10, however, had superior sensitivity to DSM-III-R. This study thus suggests that ICD-10 should be preferred for studies needing high sensitivity as well as specificity for the diagnosis of schizophrenia in the acute phase, such as studies of incidence. It also suggests that dropping the 6-month duration criterion should be considered for a future DSM-V [10].

In another study, by Van Os et al. [8], the introduction of a ''treatment-relevant'' classification of psychiatric disorders such as the functional psychoses was explored. In a sample of 706 patients aged 16–65 years with chronic psychosis, psychopathology was measured using the Comprehensive Psychopathological Rating Scale (CPRS). The principal component factor analysis of the 65 CPRS items on cross-sectional psychopathology yielded four dimensions of positive, negative, depressive, and manic symptoms. The authors concluded that although it was possible to reliably label combinations of psychopathological phenomena, the resulting diagnostic entities reveal very little about the patients. In patients with chronic psychosis, the dimensional approach constitutes a treatment-relevant alternative or complementary strategy. Its use in clinical practice, research, and service evaluation was in need of further investigation [8].

C. International Pilot Study of Schizophrenia: Symptom Frequencies in Cross-Cultural Groups

Computerized statistics have often been used to select diagnostic criteria. Such an approach will seek to select a set of symptoms that are relevant and distinct. The symptoms selected would be required to satisfy the following conditions: They should be common in a representative sample of the population under investigation. Thus, catatonia is not useful, although it's quite a striking symptom, because it is relatively infrequent; they require a high interrater reliability, and this eliminates symptoms that are difficult to identify consistently to serve well as diagnostic criteria the symptoms should be nonredundant; that is, they should be fairly independent of each other but necessary for the diagnosis (this means that they should not have high mutual intercorrelation to avoid tautology); and symptoms to be preferred should have discriminant value for the purpose of differential diagnoses, occurring quite often in concordant cases and rarely, if at all, in discordant cases with an alternative diagnosis. All these conditions define the following statistical criteria for the evaluation of symptoms characteristic of the illness: an adequate rate of occurrence, good interrater reliability, low intercorrelation of symptoms, and a high frequency ratio for concordant versus discordant groups.

Symptom frequencies in concordant and discordant groups from large-scale cross-cultural investigations

were published in the International Pilot Study of Schizophrenia (IPSS). Those data can be used to explore the potential for establishing the new diagnostic rules or criteria for schizophrenia.

The IPSS teams found that >40% of patients in concordant and <10% of patients in discordant groups had "experiences of control" (delusions of uncommon mental or physical external influences on the patients). "Auditory hallucinations" met the same conditions. If a less stringent criterion is used (at least 40% of members of concordant groups), flatness (flat affect) appears as a relevant marker. Some other symptoms (e.g., lack of insight, patient-related cooperation difficulties) appeared equally promising on the basis of their high incidence in the concordant group, but were too frequently observed in the discordant group [11].

X. FUTURE RESEARCH AND NEWER CLASSIFICATIONS

A. Reactive Psychosis: A Classical Category Revisited

Reactive psychosis was a category included in ICD-8 and ICD-9 as "reactive depression, reactive excitation, reactive confusion, acute paranoid reaction and unspecified reactive psychosis," and as "brief reactive psychosis" in DSM-III and DSM-III-R. However, in ICD-10 and DSM-IV it no longer occupies a separate category; instead it is subsumed as a subcategory in "acute and transient psychotic disorders" and "brief psychotic disorder," respectively, as "acute and transient psychotic disorder with marked stressor." ICD-10 and DSM-IV only require specifying the presence of a stressor prior to the outbreak of a usually brief, acute psychosis. However, Ungvari et al. [12] have reviewed the diagnostic concept and felt that the classical psychopathological concept of reactive psychosis goes beyond this by stipulating the temporal and contextual continuity between the stressful situation and the ensuing psychosis, taking into account the patient's personality and life history including individual vulnerability to psychological trauma. This diagnostic category featured in the psychiatric literature for several decades mostly on the basis of clinical experience. However, the original form of reactive psychosis had faded away before serious attempts were made to validate this diagnostic category. Currently the concept is not acknowledged or used in clinical practice outside Scandinavia. The wider recognition of reactive psychosis and its delimitation from other acute psychotic disorders would be important for providing clinically more homogenous samples of subjects for psychiatric research [12].

B. Refining "Acute Brief Psychoses"

One of the proposals for ICD-11 and DSM-V has been focused on the diagnostic classification of nonaffective acute remitting psychosis (NARP), also termed acute brief psychosis. The authors have suggested that this category can be delineated from both schizophrenia and the affective psychosis and be considered as a single diagnosis. They have proposed that four criteria be considered central to the diagnosis:

1. Nonaffective
2. Acute onset (over <2 weeks)
3. Recovery within a brief duration (<6 months)
4. Psychosis broadly defined.

The authors felt that both the ICD-10 and the DSM-IV lacked a firm empirical grounding in their classification of acute psychosis. Studies have indicated that the model duration of acute psychoses in the developing country setting is 2–4 months, whereas both ICD-10 and DSM-IV offered diagnoses that excluded psychoses of >1 month's duration. Furthermore, NARP is a highly distinct entity, as evidenced by studies on its demographic distribution, incidence, duration, and long-term course. The clinical characteristics are atypical for schizophrenia and affective psychoses, and it has a stable long-term course. Following recovery from the initial psychotic episodes, the cases rarely evolve into chronic disorders. Even on relapse, the subsequent psychotic episodes tend to be acute in onset and brief in duration. The diagnosis of NARP rests upon criteria, which could be reliably and comparably rated across diverse settings, and offer to bring the nosology of acute psychoses into far closer accord with current empirical data [13].

In summary, diagnostic criteria have evolved over the past five decades from vague concepts based on ideological viewpoints to field trials tested criteria which have high reliability across cultures. We have used schizophrenia as an example to illustrate the evolution of the changes in the DSM and ICD systems, but these evolutionary changes are true to a large extent of other diagnoses as well. Unfortunately, we still have to rely on signs and symptoms assessed clinically to come to a diagnosis. The lack (as yet) of biological markers in assisting clinicians to make a diagnosis can be seen as a drawback in psychiatry today. However, the rapid advances being made in