Neurochemistry and Neuropharmacology have both become interdisciplinary fields in the sense that the study of brain chemistry and the effects of drugs on the central nervous system (CNS) now require the use of methods from the fields of neurophysiology, neuroradiology, genetics, and immunology. Thus, the division of the neurosciences into specific subdisciplines has become somewhat arbitrary. These developments have emphasized the need for multidisciplinary approaches and have made more urgent the need to involve qualified basic scientists in studies of clinically relevant problems.

The Neurochemistry and Neuropharmacology Panel addressed areas of research that were felt to hold promise over the short and long term for advancing the understanding of the neurobiology of schizophrenia and its treatment. Much of its attention was focused on preclinical or basic science research. The Panel organized its discussion and report around the following topics areas: molecular biology, developmental neurobiology, neuroendocrinology and stress, neuropharmacology, primate research, imaging, and resources. The Panel recognized that the breadth of its assignment would make overlap with principal areas covered by other Panels unavoidable. In cases where this overlap was substantial (e.g., molecular biology and brain imaging), an abbreviated discussion of the topic is presented in this report with emphasis given only to areas that were felt to have particular relevance to neurochemistry and neuropharmacology. The Panel attempted to hew a line in its recommendations that pointed up specific areas of movement, promise, and importance, but without constraining the creative process or preempting the prerogative of individual scientists to develop specific projects. It is the view of the Panel that a balance should be maintained between targeting of important research goals and investigator-initiated ideas. For instance, it was the unanimous opinion of the Panel that the use of molecular biological methods could make possible new approaches not achievable with other methods. Thus, one target might be to facilitate the introduction of those methods into ongoing research programs when this would be advantageous.

### Molecular Biology

The introduction of molecular biological methods into neuroscience has opened up three new areas of opportunity for the study of schizophrenia. Significant advances have already been made, using linkage studies, in identifying the locus of a genetic abnormality in Huntington's disease, in some cases of bipolar illness, and in Duchenne's muscular dystrophy. The use of similar approaches in schizophrenia seems to hold particular promise. These studies require the identification of appropriate kinships and the collaboration of epidemiologists, clinicians, and molecular biologists. These studies are in the purview of the Genetics Panel and will not be dealt with further here.

The second approach, the study of candidate genes, is the concern of this Panel. The gene for tyrosine hydroxylase, for example, has been characterized and is of great interest for the study of the various biogenic amine systems. These studies can be carried out in autopsy material, in...
peripheral blood cells, and in living brain tissue from schizophrenic subjects, if and when tissue is available from neurosurgical procedures carried out for other brain disorders.

The ability to immortalize peripheral blood cells has provided access to living tissue in which the search for abnormal candidate genes can be readily carried out. These cells also provide an opportunity to study gene expression and regulation of those genes of interest in brain function that are also expressed in peripheral cells. The full value of this potentially important window into the brain is not yet known. This is so because it has not been established whether most genes of interest are expressed in peripheral blood cells, and for those that are, it has not been established that their regulation is the same as that in the brain. Thus, the development of more normative data on the correspondence, or lack thereof, between CNS and peripheral blood cell genes would greatly facilitate their study in mental illness.

The third approach, the use of differential hybridization, may make possible the bootstrapping of new hypotheses about the locus of a genetic abnormality in schizophrenia. This approach, in some cases, makes possible the detection of abnormal genes by comparing them with normal genes. While the potential payoff may be great, the approach must be considered to have a lower probability of success. The successful detection of an abnormal gene by this method is contingent on there being a low degree of normal polymorphism of the gene, and its expression must be at a sufficiently high level compared to other genes. Despite these serious limitations, it is the view of the Panel that some support should be given to differential or subtractive approaches because of their potential for enabling investigators to identify promising new directions for research.

**Genomic Regulation of the Dopamine System.** Given the compelling evidence linking dopamine system function to the mechanism of action of neuroleptic drugs and to the pathophysiology of schizophrenia, focused efforts should be brought to bear to use the techniques of molecular biology to study the dopamine system. Specifically, the genes for the dopamine receptor and for key enzymes influencing and controlling dopamine system function should be cloned. Efforts in this area are currently in progress throughout the country and the world, and major advances have already been made (e.g., cloning of the human tyrosine hydroxylase gene). Support for this work should be forthcoming and timely. The resultant probes can then be applied directly to clinical studies in which their link to the illness can be examined through pedigree, population genetic, or post-mortem application. There are few areas of basic science which appear as important as this for schizophrenia research at present. Once the dopamine system has been addressed, priority should be given to other CNS systems, including those that are known to influence dopamine function.

**Developmental Neurobiology**

Schizophrenia is a developmental disorder with a characteristic onset in late adolescence and early adulthood. For this reason alone, the study of developmental neurobiology is important for schizophrenia research. Developmental issues, however, are also fundamentally important in determining mature brain function. Prenatal and perinatal influences on brain development as well as later influences can affect both brain structure and function. Gene expression results from the interaction between information encoded in the gene and extragenetic regulatory factors. Extragenetic factors are diverse: maternal stress, maternal chemistry, microorganisms, environmental chemicals, and pharmacologic agents are some specific examples. Mental illness may be considered a breakdown in the ability of the brain to maintain a normal adaptation to psychosocial contingencies and is very likely reflected in aberrant adaptation at the cellular level. Better information on the role of gene structure and of extragenetic influences on prenatal and postnatal development would lead not only to a better understanding of the evolution of mental illness, but also of normal behavioral function.

**Prenatal Influences on Brain Development.** Prenatal maternal influences on the adaptation and adaptability of the organism in postnatal life has been an area that has received less study than its importance to clinical neuroscience would suggest. Immediately after conception, the principal source of embryonic RNA is from the maternal egg. Thus, the first proteins may be of maternal origin. As development proceeds, the placenta acts to transfer much selected information from mother to child. Postnatally, biological adaptive mechanisms act in concert with learned coping strategies.
to maintain stable behavioral function.

To date, most emphasis in the area of prenatal influences has been given to factors resulting in structural teratology. Subtle developmental influences on brain function may be particularly important in determining the neurobiological substrate of mental illness—it should be remembered that structural brain abnormalities seen in schizophrenic patients using modern imaging techniques are nonspecific and, by comparison with classic neurologic disorders, not severe. In the absence of observable structural teratology, emphasis should be given to prenatal factors that abnormally influence the activity of neurotransmitters, receptors, and other critical aspects of synaptic transmission. Relatively subtle, but critically “placed” abnormalities occurring during pregnancy may be produced by agents not generally considered to be noxious (e.g., antipsychotic drugs and anxiolytic agents) or by infectious agents less pernicious than those known to produce serious disruptions in brain development.

Developmental Molecular Biology. It has become increasingly likely that researchers will be able, via linkage studies, to hone in on segments of chromosomes associated with serious mental disorders such as schizophrenia and manic-depressive illness. Yet, a genetic abnormality may only be a predisposing factor; the importance, therefore, of understanding how extragenetic factors influence gene expression is clear. It is noteworthy that there are a large number of messenger RNA species that are brain specific. This may signify that many genes in the brain are regulated differently from those in the peripheral organs. The identification of regulatory proteins, associated with the developing brain, which interact with key DNA sequences may provide important clues to the expression or nonexpression of the illness-related genetic factors.

Models of Normal and Abnormal Development. Developmental models can play a crucial role in the study of mental illness, but they will require involvement of scientists at the basic as well as clinical levels. An example is a recent developmental theory for schizophrenia which proposes that a fixed, genetically influenced brain lesion interferes with normal maturational events. The effects of the dormant damage are not usually apparent in childhood because the brain structures affected do not mature or “come on line” until late adolescence or early adulthood. Key aspects of this heuristically important hypothesis are derived from the results of primate experimentation and modeling. Preclinical and clinical models focusing on critical periods of brain development and the impact on behavior deserve focused research effort.

New Strategies. Encouragement is given to the development of new strategies to get at the issue of developmental neurobiology. A number of directions were presented to and discussed by this Panel. For example, the expression of proteins that occur with synapse formation in specific brain regions should be examined using immunocytochemistry and in-situ hybridization techniques. It is necessary to know the time of appearance of key synaptic and other neuronal structures during development. In addition to information about microstructure, it is necessary to determine when each component and the system as a whole become functional. One objective of ongoing research that promises to illuminate important regulatory issues in development is the study of developmental plasticity. One key question is the extent to which structure and function are determined by the gene and the extent to which quantitative and qualitative options exist. A seminal example is the study of Patterson and his collaborators showing that certain neurons have the option of becoming cholinergic or adrenergic depending on their milieu. The way in which maternal factors influence such selective options is clearly a matter of great interest. In recent studies it has been shown that maternal drug treatment can modify brain development and function, including response to potential stressors. Studies of factors influencing development are important in understanding the brain’s response to stress, and the influence of pharmacological agents and environmental chemicals on development.

It is ironic that as developmental neurobiology becomes more molecular in its methodology, age-old questions of the relative roles of nature and nurture become more amenable to experimental test. For example, the influence of specific prenatal and postnatal stressors on expression of particular genes, important in stress response, can be directly studied.

Neuroendocrinology and Stress

Despite the compelling evidence supporting the importance of genetic factors in the development of
schizophrenia, gene penetrance does not appear to be complete. The implication is clear: environmental influence on brain and other organ function is likely to call forth the expression of the genetic trait resulting in schizophrenia. The endocrine system is one of the major links between environment and brain. Many hormones have direct effects on the brain, and conversely, the brain regulates hormone-producing systems. The hormone systems, beginning with the genomic and ending with the behavioral, are potentially important to schizophrenia research.

**Cell Factors Influencing Gene Expression.** A number of proteins mediate environmental signals and influence gene expression. These DNA binding proteins (e.g., heat shock proteins, proto-oncogenes, steroid receptors, and thyroid hormone receptors) share a common sequence of amino acids which serves as a localization signal enabling access to the cell nucleus. These cell regulators, variably responsive to extracellular influence, affect what goes on inside cells and modify or adapt gene expression in response to environmental stimuli. The modification of gene expression at the cellular level is identified as a most promising area of neuroendocrinological research.

**Brain-Environment Interaction.** The endocrine system is intimately related to brain function throughout life. Brain-endocrine communication is maintained through the hypothalamus and pituitary, and through endocrine feedback directly to the brain. This process begins early in development and is an important influence on the development of brain macrostructure and microstructure and on the maintenance of normal function. Endocrine effects early in life can influence brain function throughout the entire lifespan. Prenatal and postnatal stress, for example, produce feedback alteration in thyroid/adrenal/growth hormone output; when occurring during periods of high plasticity, these stress effects can influence brain and behavior throughout life. Sexual orientation, aging, and disease resistance, as well as the ability to adapt to stress, may all be influenced by early effects of the endocrine system on brain function. A more thorough understanding of the nature of brain-hormone interactions is likely to be of direct benefit to clinical neuroscience, in general, and schizophrenia in particular.

**Hormonal Effects on CNS Dopaminergic Function.** Specific attention to hormones (or cell modulators) that alter or modify CNS dopamine system function should be given priority. One approach is to identify hormonal events that bear some relationship to an aspect of schizophrenia and to examine their relationship to dopamine function. Two hormone groups that deserve more detailed study are sex hormones and glucocorticoids. Acute estrogen administration, for example, stimulates dopamine turnover in certain brain regions, whereas chronic treatment tends to have antidopaminergic effects. Estradiol is important in the male as well as the female because of its relationship to testosterone. The effect of sex hormones on the clinical presentation (e.g., age of onset and aggressivity) of schizophrenia deserves attention.

Glucocorticoids also influence dopaminergic activity and are of particular interest because of their relationship to stress. Steroids can produce psychosis in humans and aberrant behavior in animals—effects that are likely mediated through dopaminergic systems. An intermediate in this effect may be the opioid peptide system, which has modulatory effects on the dopaminergic system and is linked metabolically to the glucocorticoid system. Considerable advances have been achieved in understanding glucocorticoid effects of a variety of organ systems. Focused efforts to examine their effects (acute and chronic) on brain function in general, and dopamine function in particular, are recommended.

**Endocrine and Immune Relationships.** The neuroendocrine system is also involved in regulating the immune system. The thymus produces a number of hormones that reportedly affect endocrine function. It now appears that there is a substance in brain called neuroleukin which is homologous with the class of compounds called interleukins. Neuroleukins may be important to brain reorganization after an insult to the brain and may, thus, be important in enabling the brain to adapt to aversive influences. Although the implications of neuroimmunology for mental illness research are, at present, tenuous, this area of research may prove highly productive in the search for etiologies of schizophrenia.

**Stress and CNS Dopamine Systems.** The recent demonstration that the mesocortical dopamine system is, among CNS dopamine systems, uniquely responsive to pharmacological and environmental stress has
provided an interesting model linking stress and dopaminergic function. Further characterization of this model, including the duration of activation of the system produced by stress, the evidence for adaptation in response to prolonged stress exposure, and the identification of nondopamine systems that are mechanistically involved in stress activation, should be pursued. Moreover, pharmacological strategies that block or reduce activation (e.g., benzodiazepine administration) should be examined for possible clinical application to the treatment of schizophrenia.

Neuropharmacology

The introduction of the phenothiazine chlorpromazine in the early 1950's represented a landmark not only for psychiatry but for all of medicine. Several years later, haloperidol, a butyrophenone, was introduced by researchers who used information about the pharmacological effects of chlorpromazine to synthesize a more potent agent. Today, in the United States alone, there are more than a dozen commercially available compounds with proven antipsychotic efficacy and widespread use for the treatment of schizophrenia. All of these compounds, by plan, resemble chlorpromazine in their pharmacology and are referred to generically by the term "neuroleptic" (coined by the French researchers who introduced chlorpromazine to mean literally "that which seizes the neuron").

A great deal has been learned about the clinical pharmacology of neuroleptics, including the time course required for achieving optimal clinical results and the profile of their effectiveness on positive and negative symptoms of schizophrenia. Despite the truly remarkable therapeutic effects of these drugs in many patients, disabling residual symptoms are a reality for many others. Moreover, the drugs have significant deleterious side effects which, in the case of tardive dyskinesia, are at best only slightly reversible and, in the case of neuroleptic malignant syndrome, potentially lethal.

As tools for researchers, neuroleptic drugs have focused our attention on dopamine, a putative neurotransmitter synthesized and released by specific neurons in brain, and on its receptors, which exist in brain in at least two different subtypes, D$_1$ and D$_2$. The most compelling evidence linking the therapeutic effects of neuroleptics to interaction with CNS dopamine systems is the fact that the clinical potency (dose at which a therapeutic effect is achieved) of neuroleptics in treating psychosis is highly correlated with their affinity (how tightly they bind) to D$_2$ receptors in brain. For this reason, and given the fact that there is no animal model for schizophrenia, the basic science of schizophrenia research has in large measure involved basic investigations of the dopaminergic system and its innervation by other CNS systems.

Neuroleptic Mechanisms. One of the most important goals for psychiatric research is improving the pharmacotherapy of schizophrenia. Clearly, the development of new pharmacological agents that do not simply mimic the "old" drugs and provide added therapeutic effects should be given high priority. Much can still be learned, however, from studies that further elucidate the mechanism of action of currently available antipsychotic drugs. Recent electrophysiological and biochemical studies highlighting differences between the effects of acute and chronic neuroleptic administration on CNS dopamine systems functions have been paralleled by clinical studies demonstrating correlations between time-dependent reduction in plasma levels of the dopamine metabolite homovanillic acid (HVA) and clinical response. The implication of these studies is that receptor blockade is likely only one in a series of pharmacological "events" ultimately resulting in antipsychotic effects.

The importance of gaining a better understanding of the pharmacological processes involved in mediating the clinical effects of neuroleptic drugs should not be underestimated. By identifying a final rather than an intermediate step (e.g., hypothetically, "stabilization" of dopamine transmission vs. receptor blockade), pharmacological agents can be "targeted" to critical components of known antipsychotic mechanisms. Despite their shortcomings and clear need for improvement, neuroleptics likely "tap into" fundamentally important principles for the pharmacotherapy of schizophrenia.

In addition to their importance for new drug development, neuroleptic mechanisms have implications for the pathophysiology of schizophrenia. The identification of pathophysiologically distinct forms of schizophrenia on the basis of favorable and unfavorable neuroleptic response has been an influential theme of schizophrenia research over the past decade. Different etiologies (e.g., postviral autoimmune disorder, genetic metabolic error),
however, can still result in similar net CNS dopaminergic dysfunction and a clinical picture satisfying diagnostic criteria for schizophrenia. Further, dopaminergic pathophysiology that result in schizotypal psychosis need not necessarily respond in the same way or to the same degree to neuroleptic treatment. Whereas the receptor blockade model of neuroleptic action leaves little latitude for heterogeneous defect, a time-dependent model of neuroleptic action can more readily integrate a range of dopaminergic dysfunctions. Abnormal tyrosine hydroxylase activity or other genetically determined enzyme dysfunctions, for example, could diminish the critically important activation of presynaptic dopamine neurons; cortical degeneration might remove internal dopamine modulation. In both cases poor neuroleptic response would occur. Developing technologies such as positron emission tomography and molecular biological techniques should be applied to identify and/or confirm specific pathophysiological defects in CNS dopamine function in subsets of schizophrenic patients.

Typical Versus Atypical Neuroleptics. Specific attention should be given to addressing pharmacological differences between so-called "typical" and "atypical" neuroleptics, that is, neuroleptics that tend to produce extrapyramidal side effects and those that do not, respectively. In experiments using extracellular single unit recording techniques, it has been shown that typical neuroleptics (e.g., haloperidol) given chronically to rats cause a depolarization inactivation or block of neurons in the nigrostriatal, mesolimbic, and mesocortical dopamine systems of brain, whereas atypical neuroleptics (e.g., clozapine) produce this effect in mesolimbic but not nigrostriatal neurons. These data have directed attention toward mesolimbic and mesocortical structures as critical sites of neuroleptic action. The mechanisms that underlie differences between typical and atypical neuroleptic drugs are not yet fully understood. Because of their potential importance to the development of new neuroleptics that lack extrapyramidal side effects and their implication for antipsychotic mechanisms in general, atypical neuroleptic drugs should be studied intensively.

Transduction Mechanisms. A great deal of knowledge has been gained in recent years about the mechanisms that translate the neurotransmitter-receptor interaction into a biochemical and electrical response within the receptive cell. Potentially, one of these mechanisms may be the basis of depolarization block. It is known that many receptors, after binding the neurotransmitter, couple to other proteins in the membrane, called G-proteins or guanine nucleotide binding proteins. G-proteins affect the activity of a third protein in the membrane, such as adenylate cyclase, the enzyme that synthesizes the second messenger cyclic adenosine-3',5'-monophosphate (AMP) from adenosine 5'-diphosphate (ATP). There are known to exist several different types of G-proteins—Gα, Gβ, Gγ, and G0—the structures of which have been obtained through molecular cloning. Functions for all but G0 have been established, and this particular protein is found in very high levels in brain. In addition to cyclic AMP, there are a number of different compounds that are synthesized or released from within the cell as second messengers of the neuron. These include cyclic guanylic acid (GMP), inositol 1,4,5-triphosphate, and arachidonic acid. Research effort should be focused on the mechanisms whereby neurotransmitters cause the production of each of these second messengers, since these mechanisms could be sites of action of existing neuroleptics or loci for new compounds. Since different receptors can cause the formation of identical second messengers (e.g., muscarinic or serotoninergic receptor activation leads to the formation of inositol 1,4,5-triphosphate), effort should be placed on elucidation of linkages that are unique to specific receptors.

Neuropeptides. Neuropeptides have received great attention over the past decade with regard to the neurochemistry of schizophrenia and the drugs used to treat it. Although the direct benefits for schizophrenia have to date been disappointingly modest, peptide systems add important specificity for dopamine system function and for this reason remain a promising area of research.

Peptides are molecules consisting of at least two amino acids chemically bonded together in a series. Although they may represent parts of proteins, peptides can have activity on their own, and there are examples of naturally occurring, biologically active peptides containing only three amino acids (e.g., thyrotropin-releasing hormone, or TRH, consisting of pyroglutamic acid, histidine, and proline). Many peptides have been discovered in brain (hence, neuropeptides) over the past several decades and most have later been found elsewhere in
the body (e.g., the endogenous opioid peptides). The reverse is also true; i.e., peptides found elsewhere in the body (e.g., the gastrointestinal tract) were later found in the brain (e.g., cholecystokinin).

Although the exact function of neuropeptides in brain is uncertain in most cases, they may be neurotransmitters released by neurons or neuromodulators that are compounds that regulate the sensitivity of neurons to other neurotransmitters. In general, due to their rapid degradation or their molecular distribution of electrical charges, it is difficult, if not impossible, for peptides to enter the brain from the periphery—a fact that has limited attempts in humans to define the effects in brain of important peptides.

There is at least some evidence to suggest that the peptides cholecystokinin (or related peptides), neurotensin, somatostatin, and substance P are either variously abnormal in brains of schizophrenic patients or in some cases interact with dopaminergic systems with negative behavioral consequences.

Basic research is needed to understand the roles of these and other neuropeptides in the function of the brain. These studies should also include research elucidating the regulation of their synthesis, release, and degradation and how neuroleptics affect these peptidergic systems at all levels. Additionally, as molecular biologists succeed in cloning the genes for the receptors for these peptides, as well as for the dopamine receptors, thus giving us knowledge of their structures, research should be done to determine what part of the receptor binds the putative neurotransmitter. With knowledge of the active site of the receptor, researchers should use molecular modeling techniques, aided by computer, to design nonpeptidergic molecules that may represent a new class of drugs to treat schizophrenia or some of the adverse effects (mainly, motor dysfunction) of some of the currently available neuroleptics.

**γ-Aminobutyric Acid (GABA), Dopamine, and Schizophrenia.**

Clinical studies suggest that agents that enhance neurotransmission at GABAergic synapses (e.g., benzodiazepines and triazolobenzodiazepines), when administered in combination with neuroleptics, have salutary effects in some patients with schizophrenia. Moreover, therapeutic effects have been observed to include improvements in negative symptoms. Known interaction between GABAergic systems and mesocortical dopamine neurons and their stress response suggest that further attention be given to studying the neurochemical consequences of simultaneous neuroleptic/GABAergic interaction in specific CNS dopamine systems. The possibility is raised that screening techniques for identifying anxiolytic compounds might be used to identify drugs with effectiveness in treating negative symptoms.

**Pathophysiological Models.** The lack of success in the development of an adequate animal model for schizophrenia should not inhibit attempts at modeling putative neurochemical and behavioral components reflecting the pathophysiology of schizophrenia. In light of altered behavioral “tolerance” to neuroleptics shown by most schizophrenic patients in comparison with normals, it is conceivable that studies done in normal animals might fail to define critical changes in systems either by specific treatment or spontaneous responsivity. Well-designed lesion studies, however, may be useful. For example, the demonstration that lesioned mesocortical dopamine neurons are associated with enhanced subcortical dopamine function provided a critical perspective for a recent hypothesis implicating an abnormality of prefrontal cortical function in schizophrenic patients. Recently, an electrophysiological study has shown that responsivity to apomorphine in a dopamine receptive area (SN pars reticulata) is made more homogeneous by a 6-hydroxydopamine lesion of the nigrostriatal pathway. Still other studies have shown that lesions may alter the pharmacological profile of dopamine receptors themselves. Cumulatively, these data support the notion that the dopamine system is plastic and that the intactness of innervation may fundamentally alter responsivity to a variety of inputs. For these reasons, it is clear that the study of an isolated CNS dopaminergic system or systems may not be sufficient for gaining a complete understanding of the pathophysiology of schizophrenia.

Systems that innervate and bring added specificity to the dopamine systems need to be tested experimentally and their neurochemistry coupled with behavior. The techniques of in vivo dialysis and voltometry allow for the ongoing analysis of neurochemical changes in the awake freely moving animal and deserve support for their application to models of the pathophysiology of schizophrenia.

Another approach that may hold promise for understanding the pathophysiology of specific symp-
toms in schizophrenia is to study the neuropathology associated with equivalent symptoms in psychoses with established organic pathology. The study of autopsy material, as well as use of brain-imaging approaches, may give insight into the locus of disturbance in a number of symptoms shared by schizophrenia and selected organic psychoses.

The use of hallucinogen-induced states in animals as a model for schizophrenia has fallen into disfavor because of the recognition that the chemical syndromes produced by these drugs generally differ from that in schizophrenia; however, a better understanding of the mechanism of action of hallucinogens without regard to their relevance to schizophrenia would be a major advance. Knowledge of the pathophysiology of drug-induced psychosis would greatly facilitate the ability to formulate hypotheses about other psychoses.

**Primate Research**

Since reliable animal models of schizophrenia and other serious mental illnesses are not currently available, basic preclinical studies have involved several approaches in animal research: (1) investigations of the action in normal animals of drugs known to be effective in the treatment of schizophrenia (i.e., the acute and chronic effects of neuroleptics) in an attempt to clarify their possible modes of action; (2) studies directed at investigating in animals the biochemistry, physiology, anatomy, and pharmacology of chemically defined neuronal systems that may be abnormal in schizophrenia (e.g., the midbrain dopamine systems).

**Rodent-Primate Differences.** The experimental mammalian species used in the majority of these studies has primarily been rats. There is a growing appreciation that there are very important differences between rodents and primates, not only in their responsiveness to drugs but in the underlying neurochemistry, anatomy, and physiology of chemically defined neurons in the CNS. For example, while the dopamine system is evolutionarily very old in the rodent, the newer areas of the cortex such as the prefrontal and ventral regions are poorly developed. Schizophrenia is an illness with features implicating the higher cortex, in which function may be critically different in rodents and primates; moreover, relationships between the mesolimbic and mesocortical systems may be different in rodents and primates.

There are numerous differences in the biochemical regulation and anatomy of monoamine systems between primates and the more commonly studied experimental species such as the rat. Perhaps the most striking recent example is the unique potency of the neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in destroying nigrostriatal dopamine neurons in humans and monkeys, but not in rats. In fact, the initial experiments with MPTP were conducted in the rat and few toxic effects were observed in this species. Thus, the recent important research on MPTP in Parkinsonism was delayed for years until the toxic effects of MPTP were accidentally discovered in humans and confirmed in monkeys. These findings in primates led to a new animal model of Parkinson's disease.

Other important differences in catecholamine systems between primates and rodents have also been reported. These include differences in the distribution in brain of the "A" or "B" type of one of the major metabolic enzymes of the monoamines, monoamine oxidase; differences in the metabolite that is the most reliable marker of central noradrenergic activity, 3-methoxy-4-hydroxyphenylglycol (MHPG) or dihydroxyphenylglycol (DHPG); and differences in the brain metabolite that is the principal marker of central dopaminergic activity, dihydroxyphenylacetic acid (DOPAC) or HVA. In each of the above cases, human and nonhuman primates are similar to each other and different from rodents (the "B" enzyme, free MHPG, and HVA predominate in primates). In every one of these examples, the incorrect conclusion based on studies of rodents alone has been extrapolated at considerable cost to human clinical studies. Furthermore, since the direct determination in brain can only rarely be made in human studies, the perpetuation of misinformation can be long-lasting and obscure the true facts in derivative studies, cause erroneous interpretations, and result in inappropriately designed clinical studies.

Apparent differences are also emerging regarding the anatomical distribution and possible functions of endogenous peptides among mammalian species which might also explain some of the functional differences related to the midbrain dopamine (DA) systems. For example, neuropeptide is known to coexist with DA in certain midbrain DA systems in the rat, with the majority of mesoprefrontal DA neurons containing neuropeptin. A similar coexistence is not found in the monkey. In
the primate, preliminary data suggest that no neurotensin cell bodies are found in the ventral tegmental area or in the substantia nigra, while in the rat all neurotensin-containing cells in the ventral tegmental area also contain the classical transmitter DA.

**Extension of Findings to Primate Models.** It is therefore extremely important to emphasize the necessity of carrying out a number of critical neurochemical and pharmacological studies relating to schizophrenia in nonhuman primates before the general findings generated in animal studies can be extrapolated to humans. A number of important neurochemical and pharmacological findings in nonprimate species that are thought to be relevant to schizophrenia should be re-examined in a primate species to test for their validity before extrapolation to humans. It is recommended that the following important questions based on observations made in rodents be tested or reexamined and extended in research using primate species to determine if the concepts developed from these studies are relevant to man.

- Do dopamine autoreceptors play an important role in the modulation of midbrain DA function in primates? What is their distribution?
- Do all midbrain dopamine neurons have a similar pharmacological profile, or can certain subsets (such as the mesocortical DA neurons) be selectively affected by drugs or environmental stimuli such as has been observed in rodents?
- Do midbrain DA neurons in primates go into a state of depolarization-inactivation following chronic treatment with neuroleptic drugs? If so, is there a biochemical marker for this action?
  - Do benzodiazepine/GABA receptors on mesocortical DA neurons in primates play an important role in modulating their function?
  - What is the anatomy of neurotensin-containing neurons in primate brain? Does neurotensin play a role in the modulation of the function of midbrain DA neurons in primates?
  - What is the anatomy of the mesocortical DA system in primates, and what are the afferent inputs to these systems?
  - What are the regional effects of chronic neuroleptic administration on midbrain DA neurons and their postsynaptic receptive neurons with a focus on mesocorticolimbic projections?
  - Will acute or chronic environmental stress or administration of anxiogenic agents cause a metabolic activation of selective (mesocorticolimbic?) midbrain DA systems? If so, can this activation be altered pharmacologically?
  - Can the functional output of midbrain DA neurons in primates be altered by administration of precursor tyrosine in either a basal or activated state?
  - Do the pharmacological strategies that are effective in targeting selected midbrain DA systems in the rodent also effectively target the same DA systems in nonhuman primates?
  - Do functional perturbations in one DA system (e.g., the DA innervation of the nucleus accumbens) result in a subsequent alteration in other DA systems? If such changes occur, are they developmentally regulated?
  - Does ex vivo measurement of the biochemistry of central monoaminergic systems reflect the in vivo biochemistry? Can methods of in vivo assessment of central monoaminergic systems be made in unrestrained, freely moving animals in a social setting? Are monoamine measures in brain and body fluids useful indices of alterations in the activity of defined monoamine systems in the CNS of nonhuman primates? Can specific behaviors be altered or induced by selective stimulations or activations of midbrain DA systems?

**Imaging**

The growing armamentarium of relatively noninvasive techniques allowing studies of brain neurochemistry holds some of the greatest promise for furthering our understanding of the biological underpinnings of schizophrenia. Functional brain-imaging techniques can provide information relevant to (1) pathophysiological mechanisms associated with schizophrenia; (2) the mechanism of action of currently available drugs proven to be useful in treating schizophrenia; and (3) the evaluation and application of newly developed pharmacotherapeutic agents. Whereas functional brain-imaging techniques—particularly positron emission tomography (PET) and single photon emission computed tomography (SPECT)—offer the most immediate opportunities, technological breakthroughs could lead to structural brain imaging via magnetic resonance imaging (MRI) that would provide orders of magnitude finer resolution than is now possible; individual receptors could be visualized in a living human. Indeed, the field is moving toward a confluence of structure and function, physiology and chemistry.
PET and CNS Dopamine Systems. Owing to the well-documented relationship between CNS dopamine systems and antipsychotic drug action, considerable attention has already been focused on the assessment of CNS dopamine systems using PET technology. Recently, two groups of investigators have gained estimations of the numbers of D<sub>2</sub> receptors in the caudate/striatum of schizophrenic patients by using positron-emitting D<sub>2</sub> ligands. Although contradictory results were found, a fact possibly related to important differences in methodologies, these studies nevertheless represent a technological landmark in brain imaging. The critical question about abnormality in D<sub>2</sub> receptors in schizophrenic patients, however, remains to be satisfactorily answered. Moreover, a thorough examination of CNS dopamine system activity demands assessment of both presynaptic and postsynaptic DA neuronal function. A number of PET research centers are nearing the capability to visualize presynaptic DA function using positron-emitting forms of dopa. Resources needed to carry out the methodological and toxicological studies required for clinical application of this technique should be given priority. A thorough assessment of the function of dopamine systems in the human brain using brain-imaging techniques remains at present an unfulfilled but achievable goal.

PET and Neuroleptic Action. PET technologies may provide key information about the mechanism of action of neuroleptic drugs. Recent preclinical and clinical studies have suggested that neuroleptic-induced time-dependent alterations in DA activity may be more closely associated with the antipsychotic mechanism of neuroleptic drugs than receptor blockade itself. Consistent with this notion, modest (subclinical) doses of neuroleptics have been found in PET studies to result in a high percentage occupancy of D<sub>2</sub> receptors in the caudate nucleus. Moreover, it has been reported that 3–12 days are required for neuroleptics to clear a patient’s striatum once chronic treatment is stopped; relapse does not appear to parallel the loss of receptor occupancy/blockade. Other aspects of PET studies that are relevant to neuroleptic mechanisms include the identification of selective responsivity of CNS dopamine systems to neuroleptlc treatment (e.g., mesocortical vs. nigrostriatal/mesolimbic). As resolution improves, PET will provide increasing leverage for studying mechanisms of neuroleptic drug action.

Non-DA CNS Ligands. Ligands for PET and SPECT need to be developed which label non-DA receptors that are also affected by neuroleptics or are known to modify CNS dopaminergic function. Recently developed ligands have now been used successfully to visualize opiate receptors in normal subjects. Their application to schizophrenic patients is expected in the near future. SPECT holds special promise for simultaneous visualization of multiple metabolic pathways in the same individual. Creative ligand development is one of the greatest needs in the application of functional brain-imaging techniques to schizophrenia.

Task Performance and Brain Function. Both PET and SPECT make possible an understanding of shifts in neurobiological function associated with experimental cognitive tasks or with drug treatment. This is particularly important given the likelihood that the deficits in the CNS that result in schizophrenia only become apparent when certain brain areas are put “under a load,” i.e., are challenged with a specific task or pharmacological probe. For instance, studies using xenon regional cerebral blood flow have demonstrated reduced frontal activity when patients were asked to perform the Wisconsin Card Sort Test, an abstract reasoning task that requires activation of that part of the brain. A recent PET study found that unmedicated patients showed lower than normal glucose utilization in their frontal lobes when attempting the continuous performance test, a measure of auditory attention, another frontal function at which schizophrenic patients characteristically falter. After neuroleptic medication was resumed, the performance of many of the patients improved and was accompanied by increased frontal glucose utilization, linking improved behavioral function to correction of DA activity in that area. Such strategies hold promise for pinpointing the functional role of neurotransmitter systems in particular brain sites.

Animal Studies. Functional imaging techniques are used to full advantage when they are closely coupled with both primate and rodent studies. Studies using small animal species such as rodents can be done in laboratories which, while associated with PET facilities, are not necessarily physically proximate. On the other hand, for the large primates in which the PET technique can be used, the facilities of these
primates should be physically proximate to the PET facility. Large primates are probably the only group of animals which can first be studied noninvasively with PET and subsequently studied invasively to verify or interpret the data obtained by PET. Sufficient differences in the organization and chemistry of the primate and rodent brain require that primates be used as the experimental animal for certain studies.

On the other hand, certain findings emerging from PET studies can only be answered by experiments using smaller animals. For example, what does it mean that a particular drug produces a change in glucose utilization in a particular brain region? Is there increased electrical activity, or is there hyperpolarization? Is the change in glucose metabolism occurring primarily in the presynaptic or postsynaptic part of the neuronal system? Is the change in glucose metabolism occurring in the cell with the receptor that binds a given drug, or is it occurring in an interneuron or in neurons which are downstream? Why do different neuroleptics that appear equally efficacious therapeutically have markedly different profiles of glucose utilization in different parts of the brain? The answers to such difficult questions probably will require use of smaller animals.

MRI Spectroscopy. When superconductive magnets are perfected, a new generation of highly sensitive magnetic resonance spectrometers could theoretically make accessible a whole new world of intracellular metabolism; however, these developments are still not at the application stage.

Psychiatric Training. At present, few PET laboratory directors have backgrounds in psychiatry. Yet, the design of meaningful PET studies of schizophrenia requires knowledge of the complex clinical and experimental subtleties involved in such investigations. Training psychiatrists in PET and other brain-imaging specialties should receive high priority. Additionally, varying degrees of familiarity with and expertise in molecular biology has become mandatory for scientists working in the neurochemistry and neuropharmacology of schizophrenia. Conferences, work groups, and opportunities for more intensive training for selected investigators should be provided.

Resources

Despite the fact that schizophrenia is the major chronic psychiatric disease, there has been a relative paucity of resources committed to the elaboration of its neurobiology. The situation is, paradoxically, paralleled by an explosion of information in basic neuroscience. This circumstance offers great hope that it will be possible to develop a more sophisticated understanding of the neurochemical circumstances underlying schizophrenia than previously conceived. However, the sophistication of such research and the prolonged period of underfunding will necessitate a considerable investment if the potential offered by neurobiology is to be applied to schizophrenia. For example, should schizophrenia be caused by a virus, there are virtually no laboratories in any department of psychiatry in the country capable of pursuing this lead. The number of departments of psychiatry capable of conducting studies in molecular genetics akin to those that have elucidated the fundamental mechanisms of thalassemia is almost zero, despite the connection between genetic factors and schizophrenia. Similarly, the aggressive pursuit of questions necessitating imaging technologies is possible in only a handful of sites. Even in neurochemistry, where some resources have been committed, few laboratories dedicated to schizophrenia research are fully equipped and staffed with a critical mass of scientists using the most up-to-date techniques. The major exceptions are the mental health clinical research centers, which, in a few instances, have the basic resources to conduct clinical investigation with schizophrenic patients. Unfortunately, even in these centers, lack of funds to pay for the costs of hospitalization greatly interferes with the capacity of clinical investigators to study the schizophrenic patient. Thus, a substantial commitment to improve the scientific infrastructure of departments dedicated to clinical neuroscience is a prerequisite to a credible program in schizophrenia research for, at least, the rest of this century.

There are obvious implications of this astonishing absence of an infrastructure for the conduct of research on the neurochemistry of schizophrenia. Bringing a number of laboratories to a modern standard of scientific inquiry will now be far more expensive than it would have been had so many years of neglect not occurred. Not only is the fundamental equipment lacking, but in most instances there are also manpower shortages. This portion of the Panel report focuses on resources that will be required if credible research on the neurochemistry of
schizophrenia at the interface of basic and clinical science is to be accelerated and facilitated.

**Manpower.** Simply put, adequate numbers of basic and clinical neuroscientists are not available to pursue critical lines of research on the neurochemistry of schizophrenia. To entice scientists into this field, relatively stable sources of funding must be made available; if funding is unstable, scientists will simply not risk their careers in schizophrenia research. Specialized training programs should be made readily available for both M.D.’s and Ph.D.’s. To facilitate the entry of qualified scientists, training stipends should be awarded to centers of excellence in schizophrenia research and/or centers for studying the basic neuroscience of schizophrenia. Postdoctoral fellowships for basic research in preclinical laboratories intimately connected to clinical research programs are necessary. Such fellowships might perhaps be made available through the centers on the neuroscience of schizophrenia and given by the center directors to postdoctoral fellows interested in pursuing the application of basic science techniques to problems with some, albeit indirect, relevance to schizophrenia.

Fellowships should also be made available to M.D. psychiatrists interested in a 2- to 3-year dedicated laboratory experience. In order to provide the broad range of trained experts necessary for complex collaborative interdisciplinary research, a number of training mechanisms should be used. These should include an expanded Research Scientist Development Program, training programs linked to clinical research centers, and postdoctoral fellowships for training in basic science laboratories.

**Molecular Biology.** There is every expectation that the powerful techniques developed by molecular biologists will provide great insight into the neurochemistry of schizophrenia. However, the costs of establishing a dedicated molecular biology facility are substantial. Funds must be made available for relatively expensive pieces of equipment. In some instances medical schoolwide “core” facilities already have been established. However, “schizophrenologists” could be placed in a position of being offered access to core equipment only after the needs of all other investigators in the medical school have been satisfied. Thus, dedicated core facilities within, or closely linked, to departments of psychiatry and/or neurobiology are an essential prerequisite to conducting molecular research.

**Primate Research Facilities.** There are no adequate animal models of schizophrenia. The symptoms of schizophrenia appear so complex that only the most phylogenetically sophisticated species could offer a reasonable model of schizophrenia. Hence, there is a real need to validate in primates the impressions so far gained in rodent models. Furthermore, it is likely that there are unique neurochemical and behavioral patterns that can only be studied in primates. An investment in primate facilities is an essential aspect of a national program in schizophrenia research.

**Quantitative Neurochemistry.** The clinical investigation of the neurochemistry of schizophrenia inevitably depends on the availability and sophistication of various assays to measure compounds of scientific interest. Laboratories dedicated to quantitative neurochemistry, for the purpose of supporting clinical investigation, are a prerequisite to the application of the advances in basic neuroscience to the clinical setting. Thus, like core facilities in molecular biology and primatology, centralized quantitative neurochemistry laboratories dedicated to supporting clinical neuroscience are another important component of research on the neurochemistry of schizophrenia.

The requirement for sophisticated laboratory resources to support work on the neurochemistry of schizophrenia will be an evolving need. Although the resources outlined above seem essential today, it is likely that additional resources will be just as important in the future to maintain the scientific infrastructure. For example, it is already possible to envision that molecular genetic studies will logically be extended to the development of transgenic mice containing genes of particular interest for the study of systems implicated in schizophrenia. Thus, the NIMH must consider investing in facilities for development of transgenic animals, a resource of not inconsiderable cost. Undoubtedly, this is just one example of a series of circumstances that will arise necessitating an availability of capital to keep the foundation of schizophrenia research current.

Core clinical facilities that can centralize the process of patient recruitment, diagnosis, assessment, and data management are a very effective way to conduct clinical research. The availability of these functions as resources to be used by clinical studies of schizophrenia, within a given center, provide a higher quali-
ty of data at a lower cost than if each of these capabilities were developed by every single investigator within a center. Thus, the centralization of clinical research through core facilities linked to a clinical research center is encouraged. Such centers, however, should not supplant opportunities for creative investigator-initiated research.

The availability of study populations is often the rate-limiting factor in clinical investigation. Particularly problematic are the "contaminating" effects of neuroleptics on CNS neurobiology. Attaining even an approximation of a baseline condition requires exceedingly prolonged drug-free periods or the availability of first-break and/or never-medicated schizophrenic patients. To facilitate the former, some support for costs involved in a prolonged research hospitalization must be supported, as well as the staff to make possible the management of drug-free schizophrenic patients. In the case of the latter, worldwide collaboration should be facilitated, and drug-free populations made available to a broad group of investigators through appropriate collaborative mechanisms.

Most clinical research involves procedures that do not receive third party reimbursement. With increasing efforts at containing hospital costs, research conducted within the clinical setting has become progressively more difficult. Some mechanism, similar to the general clinical research center program, but dedicated to psychiatry in general and schizophrenia in particular, is a critically needed resource.

Tissue Banks. The application of molecular genetics to the clinical situation will necessitate the availability of high-quality post-mortem tissue. Groups need to be established, perhaps as a part of some of the centers for the study of the neuroscience of schizophrenia, with the capability to conduct autopsy studies. These studies need to be preceded by a detailed ante-mortem diagnosis and assessment of patients who come to autopsy. The autopsies themselves must be performed expediently and with great neuroanatomical delicacy. Ideally, autopsy studies should be guided, if not directed, by a neuropathologist.

Equally important in the application of molecular genetics to the understanding of schizophrenia will be the development of well-characterized pedigrees. Perhaps within the context of the centers for the neuroscience of schizophrenia, a family study capability needs to be developed. Particular attention should be focused on the identification of families who have been affected by schizophrenia, or schizophrenia-related disorders, over multiple generations. Furthermore, the assessment of first- and perhaps second-degree relatives should, whenever possible, not just be limited to the usual standardized assessment instruments, but also include other characteristics associated with schizophrenia-related illnesses. For example, the evaluation of backward masking or smooth pursuit eye movements would be an invaluable addition to family study data.

High Technologies. Most "schizophrenologists" would argue that schizophrenia is a disease of the brain. To these scientists, it is not surprising that as technologies for brain imaging have improved, so has the rapidity with which brain abnormalities have been reported in schizophrenic patients. Although CT scanners are relatively ubiquitous, and psychiatrists have reasonable access to such instruments, this is not the case with many other imaging devices. Nor will it likely be the case with subsequent generations of imaging technologies. It is likely that some investment in very expensive technologies related to PET scanning and MRI will prove an integral part of schizophrenia research. Not to be dismissed in this effort, however, are the manpower needs that these technologies require. A multidisciplinary team is critical to the successful operation of a sophisticated research imaging center. It is to be hoped that centers will contain fellowship positions for clinical neuroscientists (i.e., psychiatrists and psychologists) that will allow investigators with a commitment to schizophrenia to be more than just passive users of imaging facilities, but active participants in the research enterprise.

Suggested Readings


Available From NIMH

Free single copies of Special Report: Schizophrenia 1987 are available to requesters. The Special Report summarizes recent results of schizophrenia-related research. Topics covered include diagnosis, genetics, psychophysiology, biological studies, imaging, treatment, psychosocial issues, and theoretical issues. For the first time, the Special Report will also contain nontechnical summaries to make recent research findings and issues more accessible to the general public.

Readers who wish to receive a copy of the Special Report should write to the Schizophrenia Research Branch, NIMH, Rm. 10C-06, 5600 Fishers Lane, Rockville, MD 20857.