A meeting on the molecular and neurobiological basis of schizophrenia was held April 11–14, 1999 at the Banbury Center of The Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. This report is a summary of the predominant views of the participants, as perceived by the organizers. The purpose of this meeting was integrative—to bring together in a relaxed environment three dozen outstanding scientists in disparate underlying disciplines: psychiatry, psychology, genetics, neurobiology, biochemistry, molecular biology, and pharmacology. Brief talks emphasized concepts and questions rather than presentation of data. Exchanges of information and concepts provided an emerging synthesis of current and novel, even highly speculative, ideas. The reader is cautioned that the ideas, data supporting them, and data interpretations are not critiqued in this report. Nor is there much distinction made between speculations and findings that have more experimental support. The main questions and conclusions that emerged are presented in this report, which covers the following: 1) macrobiology (what schizophrenia is in terms of definition and improved diagnostics, genetics and environment, brain structure, development, and mind), 2) cell and molecular biology (defects of the expressed disease at both the membrane and nuclear levels, molecular defects of development, neuroreceptor genes and transcriptional control, and ligands), 3) therapies (current approaches, possible targets, and animal models), and 4) newer approaches (gene expression, early treatment and prevention strategies, and other problems). Two references per participant and abstracts (available from the organizers) served as a common basis.

**Macrobiology**

**What Is Schizophrenia?**

**DEFINITION OF THE DISEASE.** The clinical phenotype provides a behavioral/descriptive definition of the disease, historically based on studies by Kraepelin, Bleuler, and Kety (Andreasen et al 1999). Defining characteristics of the disease, as set forth in the International Classification of Diseases, 10th edition, and the DSM-IV, include a period of declining course with cognitive impairment. These deficits plateau in severity but often remain chronic (McGlashan 1998; Schultz and Andreasen 1999). A major sense of the meeting is that this limited focus is both stigmatizing and incorrect. Symptoms alone, although useful to the clinician in terms of predicting the course and outcome of the illness, may not be the best way to define the phenotype. There is a need for uncovering additional underlying phenotypes (referred to here as endophenotypes), which are independent of behavioral symptoms. These may serve as pathologic markers that could statistically define the illness.

Finding “markers” is difficult, because most biological abnormalities currently identified are not present in all cases, only presenting as group differences. Such markers may not be specific to schizophrenia (SZ), and may also characterize nonpsychotic unaffected relatives or those with behavioral disorders other than SZ. A challenge to the field is to define the disease biologically rather than clinically (Cancro 2000), but at present this is a difficult and potentially unrealistic goal. Lacking a tangible biological substrate, Kety has stated, “Schizophrenia is not a disease. It’s an opinion.”

Schizophrenia is often a devastating disease, but at the same time there appears to be a modest familial association with genius. For example, Isaac Newton, Albert Einstein, Bertrand Russell, James Joyce, and the recent Nobel laureate, John Nash all have either themselves, or among their immediate family, experienced psychosis. This raises the question, “Does the illness confer an ability to conceive in original ways?” This may be particularly the case for philosophy, mathematics, and physics, but other forms of creativity may also be associated with SZ. The persistence and severity of the phenotype, which is
characterized by lowered fertility, suggests that some selective advantage may be operating among those who share some of the genetic vulnerability but do not express the full-blown syndrome. What is now apparent is that genes confer a broader risk for phenotypes other than relentlessly declining chronic SZ (Schultz and Andreasen 1999).

The consensus view of the syndrome that is now emerging shows similarities with cancer models, i.e., many etiologies, no single pathophysiologic marker, many clinical forms and many levels of severity, suggestion of multiple hits before full-blown manifestation. Presently a nonpsychiatric cognitive model is being developed by diverse investigators. It emphasizes a definite, but not consistent neuropathologic substrate, resulting in dysfunction of neural circuits and fundamental impairment in cognitive neural processes, particularly memory, attention, and executive function, resulting in a loss of harmony and failure to integrate emotions and perceptions (Benes 1999; Cancro 2000; Schultz and Andreasen 1999; Selemion and Goldman-Rakic 1999; Tsuang et al 1999).

**IMPROVED DIAGNOSTICS.** Until recently, there has been no characteristic cellular phenotype available with which to diagnose SZ. Defective microcircuitry between neurons in specific brain regions may be responsible (Benes 1999; Selemion and Goldman-Rakic 1999), but techniques for detecting such lesions are not readily available. The task for clinicians is to develop physiologically based homogenous diagnostic groups, which might serve as criteria for subclassification of the disease. The field needs to come to agreement on objective tests to enable diagnostic classification based not only on symptoms but also on endophenotype. The endophenotype should have a low prevalence in the normal population and a high prevalence among schizophrenics and their relatives. Endophenotype tests were proposed (Bertolino et al 2000; Chen et al 1999; Goodman 1996; LaMantia 1999; Tsuang et al 1999).

Psychophysiologic markers of the endophenotype that have successfully differentiated schizophrenics and their relatives as a group from others include smooth pursuit eye movement and eye velocity measurements, suggesting a lesion in the medial temporal area of the brain. These endophenotypic markers may be a manifestation of the SZ brain’s inability to process and integrate over time. Deficient processing of velocity information seems to be one component that contributes to dysfunction in the initiation and maintenance of smooth pursuit eye movement disorders in SZ, and inability to suppress reflexive saccades may be a vulnerability marker. Using smooth pursuit eye movement disorders as the endophenotype, the marker was found in 50–80% of schizophrenics, 20–40% of first-degree relatives, and 8% of normal subjects (Chen et al 1999).

Electrophysiologic measurements include tests of defective prepulse inhibition, which measures the ability of a preliminary weak stimulus to diminish the response to a following stronger one, backward masking, eyeblink conditioning, latent inhibition, P50 evoked brain wave, which reflects altered hippocampal plasticity, ability to focus attention and suppress unneeded stimuli, and P300. These are markers of impaired neural systems (Bertolino et al 2000; Weinberger 1999).

 Craniofacial anomalies and minor congenital malformations have been shown to be increased among schizophrenic individuals and their relatives, compared to control subjects. These developmental markers also have been suggested as productive measures of the underlying SZ endophenotype (Goodman 1996; LaMantia 1999).

Psychophysiologic markers, electrophysiologic markers, and markers of congenital malformation have all been shown to be associated with cognitive and executive dysfunction, particularly in the areas of learning and memory. Cognitive and executive function tests can be productively employed in studies of SZ (Tsuang et al 1999).

Useful endophenotypes in schizophrenia, which can be incorporated in animal models, will consist of physiologic and anatomic characteristics, pharmacologic outcomes, and pharmacogenetic findings.

**Genetics and Environment**

**GENETIC FINDINGS.** Comparisons of adoptees, parents, and families show a major genetic basis for SZ. Clues from epidemiology include the 1% prevalence worldwide, persistence over the ages despite decreased fertility, the classic presentation in young adulthood, greater severity among males, lack of Mendelian transmission, and monozygotic concordance of “only” 40%, suggesting nongenetic factors. The occurrence of SZ in sibs, as defined to date, is lower, 3–10%. Prevalence in family members is raised by including endophenotype markers. Risk for SZ or SZ-like syndromes in offspring of affected and unaffected twins is similar. The Danish adoption studies demonstrated an excess of SZ in paternal half sibs, who share genes but not uterine environments. First degree relatives often have SZ spectrum disorders, but only a small proportion are SZ (Kety et al 1994).

Although there are clearly strong genetic bases for SZ, and in spite of enormous efforts to apply molecular genetics, no definitive genetic findings have been identified. Linkage and association studies using sib pairs and parental/affected triads have been remarkably unsuccessful in identifying causal genes, as shown by low LOD
scores, failure to replicate initial positive findings, and failure to demonstrate genes that are necessary or sufficient. Genetic linkage studies suggest several chromosomal regions that appear to contain genes increasing vulnerability to SZ, including 6p24–p21.3, 8p22–p21, 9q34.3, and 22q12–q13, but findings remain inconsistent (Cardno et al 1999; Kendler et al 2000; Schultz and Andreasen 1999). Genetic linkages to SZ differ depending on the criteria for the subpopulation chosen. With the phenotype of SZ alone there is a very small chance of getting an LOD of 3 or greater, and there are failures to replicate (Kendler et al 2000). Furthermore, inheritance is probably complex and strongly influenced by nongenetic factors. A multigenic model may apply.

As noted above, a suggested solution is to expand the phenotype to include measures of abnormal endophenotype. Such a strategy could reduce the number of false negatives and by doing so could increase the prevalence within families, resulting in greater potential informativeness in association and pedigree studies. Defective smooth pursuit eye movement has been studied in SZ pedigrees and has been linked to 6p21.3, indicating the possibility of applying expanded criteria of disease to identify SZ loci. To date using molecular genetics, no definitive genes have been identified. CAG triplet repeats are increased (McGuffin and Owen 1996), specifically at 17q21.3 (Petronis et al 1999) and 18q22 (Sirugo et al 1997). Some of the repeatedly linked regions also include genes that are functional candidates in SZ, genes that control apoptotic processes, and genes that confer retinoid responsivity. Such chromosomal colocalization suggests a method for finding underlying mechanisms in SZ and raises the further possibility of coregulation of expression of the colocalized genes (Goodman 1998).

ENVIRONMENTAL FACTORS. There is a large role for the operation of nongenetic factors in SZ (Tsuang 2000). Do environmental insults interact with genetic vulnerabilities? Brain structure is dynamic and is altered by environmental effectors. The hippocampus exhibits structural plasticity mediated through environmental stressors. In animal models, repeated psychologic stress causes reorganization of dendritic trees in the CA3 region of the hippocampus by a process that depends not only on stress hormones in the blood but also on excitatory amino acid neurotransmitters (McEwen 1999). Could repeated stress in SZ caused by an imbalance of inputs versus stored information similarly cause a cumulative deterioration of neural connections?

Considering toxins, maternal ethanol in excess could be a nongenetic factor in SZ. Fetal alcohol syndrome has been studied as a model of environmental toxic exposure in SZ. Ethanol is an N-methyl-D-aspartate (NMDA) antagonister, which causes neurologic defects and massive apoptotic neurodegeneration in developing brain (Ikonomidou et al 2000). It potentiates \( \gamma \)-aminobutyric acid A (GABA\( A \)) receptors, as do benzodiazepines and barbiturates, and competes with retinol in the alcohol and vitamin A (retinoid) metabolic cascades. The retinoid derivative, retinoic acid, is required at the proper time and place for neurodevelopment. Maternal vitamin A deficiency or excess, because of several factors, including maternal nutritional status, excess alcohol intake, or genetic abnormalities, may alter the delicate balance of retinoids required by the fetus for proper brain function and development (Goodman 1998).

Are hormone changes in the estrogen/androgen balance during adolescence factors related to onset of disease symptoms? In animal models, estrogen can activate neurogenesis in the dentate gyrus region of the hippocampal formation. Estrogens also regulate the formation of excitatory synaptic connections in the hippocampus of female rats (McEwen 1999). A difference in male/female incidence of SZ is observed. Although SZ is more common among young men than young women, the incidence rises for women over age 45, whereas for men it declines. Thyroxine is another hormonal candidate. It has a major role in regulation of differentiation (Palha et al 1997).

Parasites and viruses, interacting with immune system cytokines, may interfere with the activities of glutamate transmission and GABA function to cause neuropsychiatric symptoms (Lundkvist et al 1998). The incidence of SZ increased following the 1918 influenza epidemic. This 1918 virus was a neuroadaptive strain characterized by a different neuraminidase coat than other influenza viruses. Influenza virus infects olfactory neurons selectively and directly. The olfactory neurons are the only brain neurons in direct contact with the environment. The virus goes to the olfactory placode (LaMantia 1999) infecting postmitotic stem cell neurons and then the ventricles containing ependymal cells, causing almost total destruction of these neurons. Olfactory placode viruses can integrate into stem cell populations. Discussion focused on why the influenza virus is found only in ventricular regions in the brain. It was noted that schizophrenic patients manifest olfactory disturbances, e.g. olfactory hallucinations and anosmia.

Because the structural plasticity of the hippocampus renders it particularly vulnerable to a variety of stressors, the above factors may relate to the subsequent development of adult psychosis.

Brain

STRUCTURE. Brain structure is modified in SZ. Earlier determinations using postmortem anatomy are being
supplemented by newer methods for scanning the living brain, such as magnetic resonance imaging (MRI) and positron emission tomography (Bartlett et al 1998; Weinberger 1999). Important parts of brain for SZ include the dopaminergic pathways, including the nucleus accumbens, hippocampus, and thalamus, which is the central switchboard of the brain. Changes in cellular architecture and circuitry in the cerebral (prefrontal) cortex are seen in the disease. Hypoactivity of a widespread area of the cerebral cortex has been reported. A reduction in interneuronal neuropil, possibly due to an increase in neuronal packing density and a loss of dendritic spines, rather than neuronal loss, has been observed. These changes may result in faulty information processing (Benes 1999; Bertolino et al 2000; Goldman-Rakic 1999; Schultz and Andreasen 1999; Woods 1998).

The hippocampus is a prime candidate in SZ. Its phenotype includes a decreased density of interneurons in sector CA2, an uncoupled increase in the regulation of the GABA_A receptor–chloride ionophore complex, a neuroleptic-dose-related increase of glutamic acid decarboxylase (GAD) 65-containing terminals and a decrease of the GLUR 5, 6, 7 subunits of the kainate receptor (Benes 1999). A phenotype of decreased SNAP-25, decreased synaptophysin mRNA, and decreased GAP 43 mRNA has been replicated and is different in SZ from all other diseases involving damage to neurons (Weinberger 1999).

Schizophrenic patients as a group show excessive brain volume loss, manifest both as excessive ventricular enlargement and excessive expansion of the fluid space around the brain. Important roles for apoptotic death mechanisms resulting in excessive ventricular enlargement are postulated, affecting preferentially, but not limited to the prefrontal cortex and hippocampus (Benes 1999; Woods 1998). Because there is no excess of gliosis in postmortem tissue from SZ patients, the volume loss is not consistent with a classic form of neuronal degeneration. The idea was considered that the surrounding supportive glial cells do not appear to have a role in SZ, in contrast to neurodegenerative diseases, such as Alzheimers (Schultz and Andreasen 1999). Because excessive brain volume loss is present at the onset of overt schizophrenia, and can be found in the unaffected twin from discordant monzygous pairs, it cannot be completely explained by the effect of psychosis or its treatment (Woods 1998).

Abnormalities in motor function ("soft signs") may be a significant component of SZ. The motoric aspects of SZ, however, have not been thoroughly appreciated, so that most brain imaging studies have excluded the cerebellum, although all the major neuronal connections flow though the cerebellum and thalamus, the brain region thought to provide integrative functions (Andreasen et al 1999).

Messenger RNA studies (reverse transcription polymerase chain reaction [RT-PCR]) in postmortem prefrontal cortex segments were performed for 14 receptor- and other SZ-related genes. Except for GAD 67, their localizations and amounts were similar to normal subjects (Akbarian et al 1995).

**DEVELOPMENT.** Evidence presented at the meeting supports the theories of SZ as a neurodevelopmental illness, with initial insult in utero and onset in young adolescence. A prodrome can be observed of impaired social function, often in the presence of brain abnormalities and particularly ventricular enlargement in the absence of gliosis (Benes 1998). These findings characterize SZs as a group, but are not found in all of them. These results raise a number of questions now under study. What mechanisms can explain the findings that the first evidence of SZ structural brain disorder appears to occur around the 2nd trimester in utero, yet the disease usually does not manifest until adolescence? Does defective early embryonal structural development gradually create the late brain modulations that underlie the disease (Benes 1998; Ikonomidou et al 2000; LaMantia 1999; Woods 1998)? Evidence that SZ is a developmental rather than a degenerative disorder is manifested in developmental defects of embryonically related organs as well as in the subtle brain defects demonstrated in SZ. These are also seen in DiGeorge and velocardiofacial syndromes. Based on these observations, the SZ phenotype has been proposed to include craniofacial anomalies and both minor and some major congenital anomalies (Goodman 1996; LaMantia 1999). Developmental genes are known to play a role in the manifestation of cortical abnormalities, and more of these genes are being discovered (LaMantia 1999; Weinberger 1999).

Brain structure continually changes from embryogenesis until early adulthood; and changes of hippocampus are seen throughout life (Benes 1998; Weinberger 1999). Normal brain volume increases 3-fold from birth to 5 years. From 5 to 15 years there is actually a 20% reduction in gray matter volume due to normal neuritic pruning, but because this is more than counterbalanced by white matter expansion due to myelination, total brain volume does not reach its lifetime maximum until late adolescence. In SZ disordered apoptotic and/or pruning mechanisms operating both in utero and through childhood into adolescence may cause aberrant neural remodeling. The defective neural circuitry may result in faulty neurotransmitter balances in adolescence (Benes 1999; Woods 1998). Several studies indicate that prefrontal size is diminished in SZ, with larger ventricles and higher neuronal packing density (Selemon and Goldman-Rakic 1999). Because the dopamine projections to the medial prefrontal and anterior
cingulate cortices continue to increase during the early adult period and possibly beyond, the onset of SZ during late adolescence may be related to the ingrowth of these fibers and to the formation of mis-wired connections. This may explain in part the finding that intrinsic cortical neurons, particularly the GABA cells, are defective in SZ (Benes 1999). These findings suggest that defective macrocircuitry between interacting networks, and both positive and negative feedback loops between parts of prefrontal cortex involved in memory and cognition may be involved (Benes 1999; Goldman-Rakic 1999). The observed defects may result from early developmental apoptotic decreases in mass, altered neuronal signaling, and/or abnormal neural migratory processes. Could early defects in neurotransmission alter localized brain architecture, which could result from deficiencies in cell migration, maturation at intermediate stages, cell induction and signaling, brain plasticity, and target area innervation (Benes 1999; LaMantia 1999; Woods 1998)?

**Cell and Molecular Biology**

**Defects in the Expressed Disease**

A major goal of basic SZ research would be to describe a common pathway that encompasses the disparate findings outlined at this meeting. Evidence for common pathways comes from work on GAD 67 (Akbarian et al 1995), NMDA receptor (NMDAR) malfunction in relation to effects on both NMDAR and GABAergic neurons (Bergeron et al 1998; Olney et al 1999), and retinoid control of expression of many SZ candidate genes, including those in the dopaminergic and glutamatergic pathways (Goodman 1998; Krezel et al 1998; LaMantia 1999; McCaffery and Drager 1994; Samad et al 1997, Solomin et al 1998; Zetterstrom et al 1997). Glutamate is the major excitatory neurotransmitter in the brain. It opens a channel that permits influx of Na⁺ which depolarizes the membrane to activate rapid axonal neurotransmission. It binds to a family of surface receptors for glutamate, among which NMDAR is thought to be of importance in SZ. Glutamate excess triggers massive apoptosis. An NMDAR neurodevelopmental hypofunction model of SZ has been proposed, with a window of vulnerability during the last trimester of pregnancy (Olney et al 1999).

Effects of drugs also support involvement of glutamate receptors. NMDARs have a glycine binding site required for this receptor’s activation, and structurally related compounds D-serine and D-cycloserine, which are partial glycine agonists, slightly reduce some SZ symptoms (Bergeron et al 1998). Drugs such as phencyclidine (PCP) that produce psychosis interact with NMDAR.

N-Methyl-D-aspartate channels also allow entry of Ca²⁺, which has a variety of effects, including release of N-acetyl-aspartyl-glutamate (NAAG), a peptide high in cortical limbic pyramidal cells. Both NAAGase and the production of N-acetyl aspartate (NAA) are 30% lower in hippocampus, frontal cortex, and temporal cortex of SZ patients and unaffected relatives, correlating with lower activity of their NMDAR (Coyle 1997). Also, NAA and NAA in hippocampus decrease after puberty in an SZ (operated) rat model (Weinberger 1999). Using imaging MRI, two regions of SZ brain with reduced NAA were found. Reduced NAA is thought to be a surrogate marker for affected neurons (Bertolino et al 2000).

γ-Aminobutyric acid is a major inhibitory neurotransmitter in the central nervous system affecting excitatory interneuronal glutamate signals. An intrinsic defect in GABAergic activity is thought to be in both the anterior cingulate cortex and the hippocampus of SZ. Based on studies in rodents and humans, neuroleptics probably influence this transmitter system in a positive way, resulting in an increase of GABA terminals (Benes 1999). NMDARs on GABA neurons are more sensitive than NMDARs on glutamatergic neurons. The relative interactions of NMDAR, GABA, and glutamate with the second messenger dopamine cascade are complex and the focus of ongoing investigations (Bergeron et al 1998; McMahon et al 1994; Olney et al 1999).

Glutamic acid decarboxylase (GAD) catalyzes production of GABA. Glutamic acid decarboxylase 67 mRNA expression is decreased in the prefrontal cortex of SZ, where it may be involved in a final common pathway (Akbarian et al 1995). This result has been replicated in two independent laboratories. One could look for drugs that modulate GAD 67 expression.

With the development of atypical antipsychotics and a
shift in focus from the dopamine receptors, other genes affecting neuronal processes are of interest. These include serotonin, which has major effects on GABAergic cells and is a target for neuroleptics (see below) and synapsin, involved in neurotransmitter release of synaptic vesicles (Kao et al 1998). The current point of view is that dopamine regulation plays a major role by acting on glutamate- and GABA-regulated neurotransmission. Apoptotic processes and other processes effecting neurodevelopment are thought to be important. Not enough is known about these processes to permit any firm conclusions at this time.

Molecular Defects of Development

For the hippocampal atrophy seen in SZ, NMDARs, which modulate cell birth and cell death, and excitatory amino acids are the focus of study. Atrophy may be due to excessive excitation due to glutamatergic neurons. There is evidence of increased apoptosis, but how NMDARs provoke programmed cell death is unknown. Apoptosis destroys neurons; knockouts in mice of apoptotic genes BAX and BCL-2 upset the developmental pattern. Steroids, too, have major effects (e.g., adrenalectomy increases apoptosis, and initiation of plastic changes in the adult hippocampus requires the presence of adrenal steroids). Progesterone causes disappearance of NMDARs. During estrus and by giving estrogens, synapses are made and replaced in the CA1 region. Here as well, underlying mechanisms are not known (McEwen 1999).

Brain plasticity has a major role in relation to neurotoxic damage in hippocampus. Importantly, adaptations of neural plasticity can be very long term. Repeated neuronal perturbations cause changes that persist for months in specific brain regions. Environmental enrichment increases spatial learning, by increasing cell survival and neurogenesis. One molecular explanation for this long-term effect is proposed to depend on a modified form of Delta fos-B, which acts as a “molecular switch.” It forms an AP-1 binding complex with Jun-D that is stable for a long time in specific brain regions. Target genes include glutamate receptors. Cocaine stimulates overexpression of Delta fos-B in striatum, and chronic administration of antidepressants causes it to accumulate in cortical regions. In transgenic mice, Delta fos-B can be induced in specific brain regions, and this increases the response to cocaine (Nestler et al 1999).

Abnormal neuronal development was seen in 30% of SZ samples. Some neurons are missing in prefrontal cortex. With evidence emerging that supports a neurodevelopmental model of SZ, one should look further at genes that affect neurodevelopment. Genes that have been proposed, and in some cases studied, include retinoid receptors, neural cell adhesion molecule (NCAM), n-cadherin, and the neurotrophins, some of which produce primary neurodevelopmental defects resulting in secondary dopamine involvement. Neurite apoptosis and pruning has been proposed to involve the neurotrophin, brain-derived neurotrophic factor (BDNF), which has a role in eliminating inappropriate neurons. It is tightly regulated by second messengers, and its mRNA is lower by 25% in SZ. A polymorphism has been identified in the promoter of BDNF in a Canadian SZ sample. Will decreased BDNF make the hippocampus vulnerable? Are there protective factors that characterize the non-SZ genotype? Is there a way to look for such protective factors (Petronis et al 1999)? Reelin is decreased in SZ. It is preferentially expressed in GABAergic neurons in cortex and hippocampus.

Neuroreceptor Genes and Transcriptional Control

An area of growing importance in SZ research is factors affecting the transcriptional regulation of neurotransmitter receptors.

MEMBRANE NEUROTRANSMITTER RECEPTOR GENES. Production of membrane-associated receptors, such as for dopamine, is regulated by ligand binding to nuclear receptor motifs in the DNA of promoters of genes coding for these neurotransmitter receptors. Conversely, there are connections between cell surface neurotransmitter receptors and nuclear transcriptional receptors, e.g., interaction of the dopamine cell surface receptors with the retinoic nuclear receptors (see below). Modification of the splicing of D2 receptors may be important in SZ. Reports of knockouts of nuclear receptor genes, including those involved in dopaminergic expression (Krezel et al 1998; Zetterstrom et al 1997), may add to our knowledge regarding the underlying phenotype in SZ.

NUCLEAR RECEPTOR GENES. Transcriptional regulation of receptors for retinoids, thyroxine, and also unknown ligands is being investigated, particularly the effects on dopamine receptors (Krezel et al 1998; Samad et al 1997; Zetterstrom et al 1997), and of the downstream genes involved, in the dopamine metabolic cascade; e.g., G-coupled proteins, adenylate cyclase, cAMP, and DARPP-32 (Greengard et al 1998; McMahon et al 1994). Retinoic acid, the ligand for the retinoid nuclear receptors, stimulates transcription of the D2 receptor, and the promoter of the D2 receptor contains a retinoic acid response element (Samad et al 1997). The retinoic acid receptor (RAR)β and retinoic X receptor (RXR)γ localize to the striatum with D2 receptor and D1 receptor (Krezel et al 1998).
Insights into gene functioning, receptor genetics, and transcriptional control in vivo are beginning to be provided by knockout mice, in which one or both alleles of a selected gene has been deleted. Both D2 receptor homozygotic and heterozygotic mice have decreased locomotion, rearing, etc. Double knockout RARβ/RXRγ have poorer rotodod performance. Mice lacking retinoid receptors display reduced D2R expression in the nucleus accumbens and drastic neuromotor behavioral impairments (Krezel et al. 1998). Retinoid receptors are essential for the long-term potentiation and depression effects of neuronal plasticity on learning and memory.

Orphan nuclear receptors with no known ligand, e.g., NURR1, appear to be important in brain functioning and adult brain disorders, as suggested by behavioral phenotypes of knockout mice. Lack of migration of dopaminergic neurons to more lateral destinations is an early characteristic in NURR1 knockouts. NURR1 acting with RXR is essential for generation of dopamine neurons of striatum and substantia nigra. NURR1 knockout mice lack mesencephalic dopamine cell markers. The homozygotic −/− mice do not survive birth; there is premature apoptosis in their brain cells (Solomin et al. 1998; Zetterstrom et al. 1997). Aldehyde dehydrogenase 2, which is expressed even before NURR1 in the innervation of dopaminergic cells, is involved in the generation of retinoic acid (LaMantia 1999; McCaffrey and Drager 1994; Yamamoto et al. 1998) and is absent in NURR1 knockouts (Zetterstrom et al. 1997), again indicating that retinoids are involved in the transcriptional control of dopamine-expressing neurons. Drastic reduction of D2 receptor expression has been demonstrated in RAR and NURR1 knockouts that completely lack these receptors, with dopaminergic neurons in the nucleus accumbens showing strong downregulation of D1 and D2 receptors. In line with these findings, cocaine had no effect on locomotion in retinoid double receptor knockouts, supporting a specific effect of these nuclear factors on the dopaminergic pathway (Krezel et al. 1998).

Evidence is beginning to accumulate associating thyroid hormone level variations with SZ. The thyroid hormones regulate the expression of lipoprotein lipase, a gene strongly expressed in the brain, which affects retinoid and fatty acid (e.g., omega-3) metabolism (Goodman 1998). The chromosomal location of the marker, lipoprotein lipase, at 8p22 shows significant linkage to SZ (Kendler et al. 2000). Transcriptional regulation of thyroid hormone receptor genes, and thyroxine are being investigated, because they play crucial roles in development of the nervous system. Transport of thyroxine and retinol binding protein from blood to brain across the choroid plexus is accomplished by transthyretin. Tyrosine hydroxylase and myelin expression, GABA release, and retinoid receptor expression are regulated by thyroid hormone (Palha et al. 1997).

LIGANDS. The action of nuclear receptors (e.g., RARs, RXRs, peroxisome proliferator-activated receptors (PPARs), NURR1, thyroid hormone, progesterone, androgen, estrogen receptors) are due to the specificity of their dimerization and ligand binding in particular target tissues. NURR1, the PPARs, and thyroid hormone receptors each partner as heterodimers with RXR and interact specifically with their ligands. This common finding suggests a control mechanism that might prove effective for developing drugs against SZ, targeted to ligands that bind to RXR (Mukherjee et al. 1998). Synthetic compounds can be manufactured to bind specifically to these receptors and can be used as probes to study their action and signaling pathways (Devchand et al. 1999; Eichele 1999).

The retinaldehyde dehydrogenases possess unique features that render them plausible candidates for creating SZ pathology. Expression of retinaldehyde dehydrogenase is limited to specific sites in neurons, involving pathways to the striatum, and in embryos to structures that give rise to the striatum and to GABAergic neurons throughout the cerebral cortex. This process continues throughout adulthood (McCaffery and Drager 1994; Yamamoto et al. 1998). Retinoids might exhibit both early and late roles in the disease. Regulation of brain development, as early as in the first trimester, is dependent on retinoic acid production by neural crest related mesenchymal cells. These cells, which are laid down early, are involved in the subsequent retinoic acid signaling activity controlling the induction of later developing cells (LaMantia 1999).

There is evidence supporting the role of 9-cis retinoic acid, the RXR ligand, in SZ, rather than the RAR ligands. The mild congenital abnormalities produced by 9-cis retinoic acid are quite specific to the craniofacial region, whereas the RAR ligands, Accutane, 11-cis and 13-cis retinoic acid, produce more severe and generalized defects (R.A. Heyman, personal communication, April 1999). This may be an important clue for SZ, since a major extension of the phenotype would be to include craniofacial abnormalities (Goodman 1996; LaMantia 1999). The milder defects and the predominance of craniofacial (rather than generalized somatic) defects implicates the RXRs rather than the RARs in the etiology of SZ. The replicated genetic linkage findings at 6p21.3, the RXRβ locus, and 9q34.3, the RXRα locus, supports involvement of the RXRs in SZ.

Therapies

Current Approaches

The typical antipsychotic drugs, e.g., haloperidol, block the activity of dopamine receptors but fail to improve the negative symptoms of flattening of affect and social withdrawal, and virtually all have unwanted side effects (Iversen 1999). The atypical antipsychotics now utilized
can retain antipsychotic efficacy, have effects on negative symptoms and cognitive functions, and are less liable to cause unwanted side effects. They have high blocking affinities for both dopamine and serotonin receptors and may function differently because of this dual effect. Promising approaches are currently to develop high affinity antagonists of both dopamine and serotonin receptors. Phospholipase inhibitors, neurotensin antagonists, and cholecystokinin and glycine effectors are also in development (Bartoszyk et al 1998; Bergeron et al 1998; Iversen 1999; Ohno et al 1995; Mondadori et al 1996).

**Possible Targets**

Differentially expressed markers, known and unknown, offer clues to new drug targets. In a complex illness like SZ there is no single target; the problem is to select a useful target. Appreciation of the generalized mechanism of ligand activity opens a new approach to drug development; every known ligand provides a possible drug target (Mukherjee et al 1998). Agonists, antagonists, and receptors that are current targets of interest include the NMDARs, retinoic acids and receptors, fatty acids, serotonin, noradrenaline, nicotine acetylcholine, and thyroxine. By understanding dopaminergic and glutamatergic signaling, it should be possible to identify new targets. Metabolism of these targets could alter or regulate the activities of a variety of downstream genes. Understanding the activity of clozaprin on gene expression might develop new targets and new candidates for drugs in SZ. Drugs could affect other neuromodulators—nicotinic acid, GAD expression and function, GABA, etc. Antagonists and agonists to these targets have not yet proven to be clinically effective, although work with glycine analogs appears promising (Bergeron et al 1998; Cancro 2000). Looking for predictors of drug response will create pharmacogenomics as a major field. In these endeavors, industry needs help from academics.

A potpourri of innovative and inventive drug strategies were discussed, reflecting the breadth of disciplines, much outside the field of neuropsychiatry, represented at the meeting. Drug development in other fields has utilized mechanisms that can modulate recruitment of cofactor proteins for signal transduction and plasticity. Alteration of the specificity of receptors is accomplished by developing new classes of compounds that recruit different cofactors, by making them cell specific or tissue specific, by employing unique transcriptional pathways, or by having unique transcriptional coactivators or nuclear heterodimeric partners. These approaches offer the potential to integrate and coordinate several transcriptional factors (Devchand et al 1999; Schulman et al 1998). DNA elements that control gene expression and imprinting by DNA methylation and histone acetylation/deacetylation participate in these mechanisms and could provide drug targets (Petronis et al 1999; Popendikyte et al 1999). Drugs that modify retinoid regulatory functions are being tested against cancers because of their differentiating and apoptotic effects. These therapies are directed against transcriptional activation by RAR and RXR ligands, known to recruit different cofactors to enable differentiation. Combinations of two such drugs gives an additive effect.

**Animal Models**

What are the best animal models? Suitable simple animal models are important for drug discovery through preliminary testing. With no really appropriate models for either the psychotic or negative symptoms of SZ, better animal models will evolve as we learn more about the disease and its molecular basis (Chen et al 1998). It was argued that animal models can only be based on molecular findings. A good animal model requires a suitable molecular assay, and conversely, discovering suitable molecular assays would be greatly facilitated by a suitable animal model. Because for SZ there are no consistent molecular findings, the field should first concentrate on refining the molecular studies.

Rats and mice are currently used to study drug effects. Surgical and chemical lesions can be introduced to produce SZ-like syndromes (Weinberger 1999). The PCP-induced psychosis model is of interest for SZ. Mutant mice, such as reeler, frizzled, and disheveled, may give clues and be useful for drug testing. Knockout animals are now beginning to be used in the study of expression of genes involved in SZ, e.g., NURR1 and retinoid receptor (Krezel et al 1998; Samad et al 1997; Solomin et al 1998; Zetterstrom et al 1997). Knockouts of both demonstrate extensive effects on the expression of dopamine receptors. A combination of increased lateral ventricle size and reduced prepulse inhibition of startle has recently been demonstrated in NCAM-180 knockout mice, and this suggests an interesting approach to an animal model of SZ. Mice expressing low levels of NMDARs appear promising.

What symptoms can be used as endpoints of drug effects in animal models? Behavioral endpoints include conditional avoidance response, and anxiolytic action (Ohno et al 1995). Catalepsy has been applied as a physiologic test model of negative symptoms (Bartoszyk et al 1998). Atypical D2 antagonists cause Delta c-fos induction, and blocking action at D2 receptors was tested by striatal fos mRNA expression (Nestler et al 1999). Altered dopamine turnover was also used as an assay (Chen et al 1998).
Simpler animals in which neural system mutants have been discovered, e.g., *Drosophila* and *Caenorhabditis elegans*, should have utility for at least preliminary testing of drug effects. Zebrafish eye tracking is being investigated (Li and Dowling 2000). At an even simpler level, cortical stem cells in culture could be used. Molecular markers in cell lines and biopsies might be applied.

**Newer Approaches**

**Gene Expression**

How are genes manifested in neural circuitry of brain? The problem is complex, but we must start. Finding the modified genes was stated to be the only approach to discovering the fundamental nature of SZ (Watson 1993).

Expression genetics will provide clues for SZ. By some estimates, more genes, perhaps 40,000, are expressed in brain than in any other organ, perhaps because diverse parts of brain function differently. Two independent estimates were made of about 300 such gene expression changes in SZ, both positive and negative. Methods utilizing functional genomics to find disease-involved genes are developing rapidly. For this purpose, the differential display RT-PCR method is currently being applied by industrial and basic scientists to discover changes in gene expression patterns in brain under various conditions (Pardee and McClelland 1999). New technologies of DNA dot displays and chips are being developed, and should soon accelerate these searches. These gene fragment arrays are now beginning to be applied mainly for detecting expressions of known DNA sequences.

At this early stage it may not be important to understand the causative factors, but rather to identify markers of change and then to go back to attempt to understand and analyze the upstream and downstream pathways in which they are involved (Akbarian et al 1993, 1995). This approach has been fruitful in studying neurodevelopment (Albrecht et al 1997). The more difficult question is to determine the biochemical function of such genetic markers. Understanding of these functions could lead to the discovery of unsuspected metabolic regulatory pathways involved in SZ and to novel drug development to alter the pathologies characterizing these new pathways.

Visualizing expression patterns of known genes in different brain regions is a further step. The task is to determine regional differences in gene expression in space at the cell level and in time. A search for novel expressed genes in SZ showed a specific increase in ventral striatum of an AMPA receptor subunit, GluR2 (Akbarian et al 1995). A molecular map of fine structure gene expressions in particular brain regions, and its alterations in pathologies, could emerge. Scanning the postmortem brain for gene expressions is commencing, applying an automated in situ approach on antibody-stained slides. These studies could provide molecular markers as well as causal changes in SZ. Appropriate protein markers are needed for this research, which requires postmortem SZ brains. Detection of expressed genes and proteins during embryogenesis of brain is being investigated (LaMantia 1999; Yamamoto et al 1998). Here a good animal model is essential (Chen et al 1997; Swindell et al 1999).

**Early Treatment and Prevention Strategies**

Can treatment block the early development of structural lesions, or the progression to disease involving excessive brain shrinking and apoptosis?

Drug treatment has already begun in cohorts of two or more affected first-degree relatives not showing SZ but demonstrating reduced brain volume in the basal ganglia and medial limbic areas (Tsuang et al 1999). Psychotic symptoms in a schizophrenic individual can appear 1–2 years before hospitalization, and change with time. Timing of the initiation of the deficit syndrome is within the first 6 months to 5 years, often prior to the onset of overt psychosis. The longer the duration of untreated psychosis, the longer to remission. Early medication in the period prior to full-blown disease when plasticity is still possible may help to decrease disease duration. There is suggestive evidence that earlier intervention will lead to better outcome. Although the reduced duration of untreated psychosis results in an increase in schizophreniform early stage illness, the incidence of full-blown SZ is lower. Whether early treatment will make a difference in the duration of treated psychosis can be tested (McGlashan 1999). Treatment for primary prevention can use novel atypical neuroleptics. An important question in this regard is whether an initially effective drug might create long-lasting deleterious side effects (Tsuang et al 1999).

The question then arises as to who should receive early intervention. This question brings us to a topic discussed earlier in this meeting: namely, identification of the molecular markers predisposing to the disease. It is not unreasonable to expect that one will be able to characterize groups or combinations of genes, mutations of which accurately predict later onset of the disease. These genes may involve those discussed above in terms of downstream involvement in neurotransmitter cascades or signaling, chromosomal location, and/or relation to the processes of apoptosis, neuronal migration, neurotransmitter expression, methylation, and growth control. For the future, early detection and treatment of this devastating disorder should be possible if suitable combinations of physiologic, anatomic, genetic, and/or immunologic markers can be identified.
Other Problems

Nonscientific problems of research and drug development are formidable. They include costs and funding at every level, ranging from research science and government grants to industry. The same problem of lack of funding was raised in summarizing the 1993 Cold Spring Harbor conference. But spending money will not help without good ideas. The field in general needs a breakthrough for getting adequate funding.

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References


