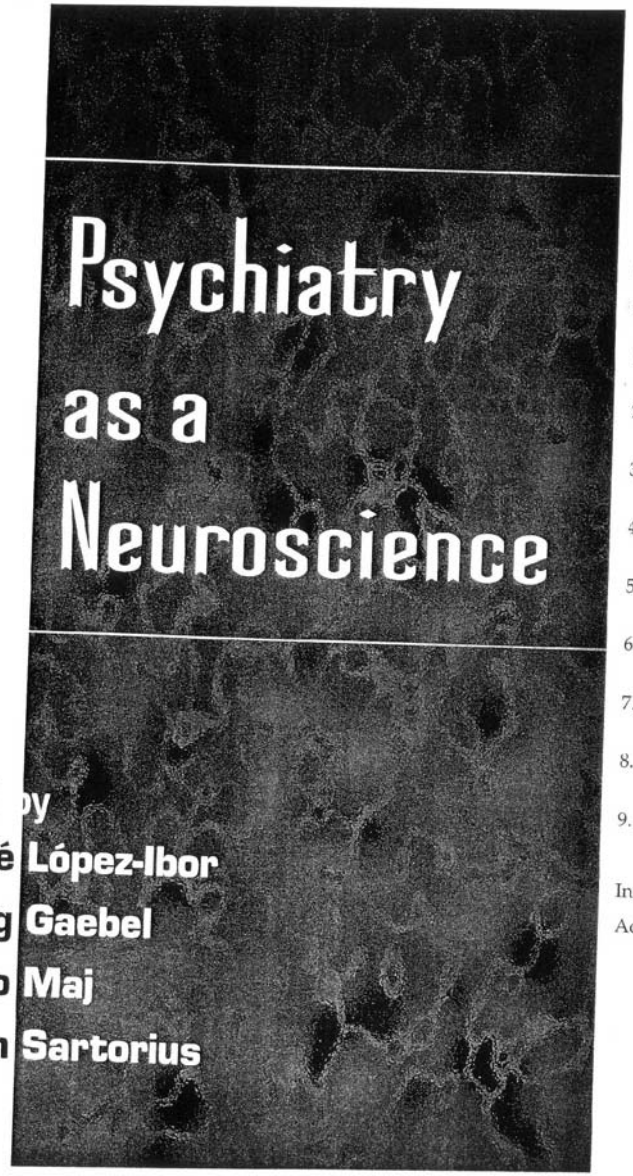

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Molecular and Cellular Biology Research in Psychiatry

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INTRODUCTION

Paradigm shifts and new technologies are revolutionizing psychiatry at the dawn of the new millennium. Psychiatry, much beholden to Freud in the past, is finding a new patron saint in Darwin. Freud himself, if his early career as a neurologist and scientist is any indication, might very well have chosen to be a molecular neurobiologist were he alive and active today. We will attempt in this chapter to provide a highly selective view of current and future trends, rather than an exhaustive overview of the data accumulated so far, and integrate it into the larger clinical picture, in the hope that this will provide the reader with a useful framework for understanding the rapid evolution in the field.

Paradigm Shifts

Clinical Comorbidity Underlined by Overlapping Biological Mechanisms

The growing understanding and appreciation of shared genes and overlapping molecular and cellular mechanisms on the one hand, and of the global impact of psychiatric syndromes on the brain on the other hand, provide a potential biological explanation for clinical comorbidity as the rule rather than the exception. It may also provide a basis for future more precise, mechanism-based classifications of psychiatric illnesses, rather than the current descriptive complexity of the DSM-IV.

Psychiatry as a Neuroscience. Edited by Juan José López-Ibor, Wolfgang Gaebel, Mario Maj and Norman Sartorius.

Tissue Remodelling

The brain is being increasingly viewed as just another organ, only more complex. The structural and functional plasticity of the brain, not unlike that of a muscle, is beginning to be appreciated [1]. The molecular and cellular changes in neural function that are produced as adaptations to chronic administration of addictive drugs, such as psychostimulants, and therapeutic drugs, such as antidepressants, have been proposed as a basis for their long-term effects on the brain, and for the latency of their therapeutic actions [2]. Moreover, the role of cell proliferation and cell death in psychiatric disorders is coming into focus [3, 4], prompting analogies with, and benefiting from concepts and techniques from, cancer biology [5, 6].

Glial cells, long thought to play mainly a supportive role to neurons, are increasingly identified as active players [7]. Post-mortem studies in mood disorders indicate altered numbers of neurons and glial cells [8]. Glial cells but not neurons were reported to be reduced in the subgenual prefrontal cortex in mood disorders [9].

A new concept that may inform psychiatry in the coming years is that of cumulative end-organ damage, in this case of (different regions of) the brain. The analogy to make is with diabetes, where cumulative glycosylation of artery walls and other tissues leads to retinal, kidney, neuropathic and other damage. This paradigm may explain why, for example, a medication tried earlier in the course of the illness may not work as well or at all when tried again later.

Endocrinology and Psychiatry

The brain, just like any other organ, is constantly exposed to and regulated by the hormones in the internal milieu, as a way of integrating its activity with the rest of the organism. The powerful influence of hormones on neuronal activity is an area that has received and will continue to receive increasing scrutiny, and the wealth of clinical observations from classical endocrinology regarding changes in mood and cognition in endocrinological disorders is now being revisited at a molecular and cellular level. Furthermore, not only the extremes of endocrinological pathology but also the physiological variations in hormonal levels are emerging as being of importance in modulating psychiatric syndromes.

Peripheral Molecular Markers

At present, diagnosis in psychiatry relies mainly on descriptive behavioural and symptomatic information. Measurable peripheral molecular markers

are being actively pursued, and may enable simpler, more rapid, more objective and more accurate diagnosis and monitoring.

Infectious Aetiologies for Psychiatric Disorders

This interesting emerging area of research holds promise in identifying endogenous retroviruses, integrated in the genome and hereditarily transmitted, as important in the pathogenesis of a subset of psychiatric disorders [10]. Another avenue being followed is that of infectious agents as "second hits" that may lead to overt disease development in individuals with an inherited genetic susceptibility [11, 12]. The parallels with cancer biology are intriguing and may be methodologically fruitful.

Concerted Approaches to a Problem

It is beginning to be appreciated that a successful way to understand mental disorders is to use and integrate different approaches concurrently: phenotypical assessment, pharmacological studies, animal models, molecular and cellular biology, genetics and brain imaging [5, 13]. At the same time, strenuous efforts are being made in the field to perfect each approach as much as possible.

METHODOLOGY

Overview

Rapid progress was made during the past decade in several important areas relevant to cellular and molecular research in psychiatry. They include a better understanding of the neural circuitry involved in mental disorders, the cloning of complementary DNAs encoding important molecular targets of drugs, including the whole extended family of dopamine and serotonin receptors [14], and progress in understanding the molecular basis of long-term adaptive processes and their role in illness progression.

New methodologies that are revolutionizing psychiatry, as they are other fields of medicine, are molecular biology, genomic research, and combinatorial chemistry coupled to rational drug design.

Molecular biology has led to the development of powerful techniques such as the polymerase chain reaction (PCR) and DNA microarrays, like the so-called GeneChips (Affymetrix Inc., USA). It has also led to the possibility of engineering gene deletions or insertions in experimental animals, the

most notable example being transgenic mice. The human genome project has been identifying the sequence of the complete set of genes, estimated to be over 30 000, variants of which are associated in different individuals with different illnesses. The number of proteins is estimated to be ten times as high, about 300 000, since a gene can encode for multiple proteins by its subsequent "tailoring" and processing.

Combinatorial chemistry has increased the pool of compounds that can be generated and tested against a specific protein target, and rational drug design based on structural imaging and modelling of proteins speeds up the drug discovery process by providing specific key-in-lock parameters for lead compounds.

Further on the horizon, neuronal stem cells may lead to transplant approaches for those mental disorders where cell loss is involved, as is currently attempted in neurology for Parkinson's disease, for example. Also on the horizon is the possibility of gene therapy, mostly for now in an early development stage and used as a research tool in animal models [15], but currently moving towards clinical applications in humans in at least one area, Alzheimer's disease (AD) [16, 17].

Gene Expression and Microarrays

The ability to simultaneously assess the expression of thousands of genes with microarray technology is opening new vistas in basic neurobiological research in both animal models and human post-mortem tissue samples [5, 18–20]. While gene expression is just part of the story of what is mechanistically happening in specific brain regions, it is an important first step in terms of identifying targets for more detailed studies, as well as for pharmaceutical drug development. More recent approaches have added the same massive parallel approach to protein assays [21] in the emerging field of proteomics [22]. The ultimate goal is the physiome, where molecular changes are integrated at a whole-organ or system level [23].

Animal Models

Obvious technical and ethical limitations dictate that studies on molecular changes in mental disorders cannot be performed on live human beings. Animal models of neuropsychiatric disorders are being developed in species as diverse as monkeys [16], rats and mice. While careful attention to animal welfare and minimizing pain and distress is a must, and ethical discussions of conducting research on animals are warranted, one should bear in mind that the ultimate goal is a worthy one, at least from our species' viewpoint—understanding and curing illness.

Monkeys are closer to humans, but more difficult and expensive to breed and conduct research on. Rats and mice are less expensive to breed and have faster generation times. Rats have bigger brains than mice, but less work has been done on creating transgenic rats. While mice have smaller brains, they are extensively used in genetic research due to their amenability to transgenic manipulation of their genome. Useful models of human psychiatric illnesses have been generated in this way [24].

Functional Imaging

Functional imaging is more developed in humans, due to a diagnostic medical impetus from fields like neurology and neurosurgery, although studies in psychiatry have yet to take full advantage of the spectrum of technologies currently available [25]. Imaging of primates and even rodents (rats, mice) is gradually being developed and standardized and may benefit basic research significantly, especially in concert with molecular genetics in well-defined animal models of disease.

Convergent Functional Genomics

The intersection of multiple lines of evidence upon a gene or biological mechanism for a particular disorder—what we would term *convergent functional genomics*—can be a powerful discovery engine. It may also reduce the uncertainty inherent in individual methodologies or lines of evidence. An approach to bridge insights from animal models and human genetic and brain imaging models has been described [5]. The basic idea is to use data from brain imaging studies to select brain regions of interest in a specific mental disorder and analyse gene expression patterns in those regions in post-mortem human brains or a germane animal model. The next step is to integrate human genetic linkage data with the gene expression data. This is done by seeing whether the genes that show a changed pattern of expression in the animal model or post-mortem human brains (compared to controls) map to linkage hotspots from human family tree genetic studies. This approach, which combines imaging, animal models and molecular genetics, is arguably quite useful in terms of identifying specific candidate genes involved in a particular mental disorder. Those genes are then the targets of extensive investigation, and the protein encoded by them becomes a potential target for pharmacological drug development.

Pharmacological Studies

Drug development in psychiatry is progressing at a particularly rapid pace, especially in view of the relative paucity of the pharmacological armament-

arium available to psychiatrists until recently, compared with other fields of medicine. This can make even a recent psychopharmacology textbook an out-of-date tome gathering dust on a shelf. We will outline some of the interesting current insights from pharmacological studies, with a clear feeling that this may be one of the more rapidly obsolescing parts of this chapter.

For at least two reasons, one has to be careful in extrapolating the results of clinical trials, with their very particular enrolled patient populations, to patients at large seen in primary psychiatric practice [26]. First, patients enrolled in clinical trials may have a more severe and refractory form of the illness. Second, due to the exclusion criteria, patients may lack the comorbidities that are the rule rather than the exception in the general patient population.

Matching a drug to a given patient in psychiatry has been and still is largely a trial-and-error process, with broadly acting drugs being administered to a large range of patients with a broadly descriptive disorder. Determining whether a given patient will respond to a given drug, or whether a given patient will tolerate a given drug, cannot be done in advance of an empirical trial. In the future, pharmacogenomic profiles are expected to predict particularly those patients who are unlikely to tolerate a given drug, and may even be able to define in advance those most likely to respond successfully to a given drug. Thus, future drugs may be indicated not for broadly descriptive mental disorders defined in the DSM-IV, but for behavioural syndromes associated with a given genotype.

Drugs serve not only therapeutic purposes, but also as probes unravelling pathophysiology, due to our still incomplete knowledge of brain function and somewhat empirical black-box approach to psychopharmacology. While the information garnered this way is no doubt useful, this chapter, and in fact the whole volume, outlines approaches that will make psychopharmaceutical research less of a hit-and-miss proposition.

SCHIZOPHRENIA AND OTHER COGNITIVE DISORDERS

Clinical Comorbidity Underlined by Overlapping Biological Mechanisms

Disorders of cognition have traditionally been classified into psychoses and dementias. Other disorders of cognition include delirium and amnestic disorders. Psychoses were deemed to occur at an earlier age, to be biochemical/genetic in nature and to be at least partially reversible pharmacologically. Dementias were deemed to occur at a more advanced age, be degenerative in nature and to be mostly irreversible. The prototypical examples of psychoses were the schizophrenias, and the prototype dementia was AD. These distinctions are becoming strained and blurred as we

learn more about the underlying pathophysiology, nowhere more so than in geropsychiatry. It is now being increasingly recognized that degenerative changes underlined by cell loss occur in schizophrenias, and that partially correctable biochemical neurotransmitter abnormalities occur in dementias. Moreover, other mental disorders such as mood and anxiety disorders, in addition to neurological disorders such as Parkinson's disease and Lewy body dementia, have an impact on cognition, with psychotic or pseudodementia-like end results.

Tissue Remodelling

The occurrence of macroscopic and microscopic end-organ brain damage in dementia as well as in schizophrenia has been appreciated for some time, more so than for other psychiatric syndromes. There is increasing evidence that the schizophrenic disease process begins before psychotic symptoms become overt [27]. These findings may also explain the limited functional recovery produced by even state-of-the-art psychopharmacological treatment, and suggest that early intervention, perhaps with agents that prevent cell death or promote cell growth, may be a valid strategy in the future.

An interesting line of work in the realm of brain plasticity has been the exploration at a molecular level in experimental animals of the effects of nurturing maternal behaviour [28]. The authors report that variations in maternal care in the rat promote hippocampal synaptogenesis and spatial learning and memory through systems known to mediate experience-dependent neural development. Thus, the offspring of mothers that show high levels of pup licking, grooming and arched-back nursing showed increased expression of *N*-methyl-D-aspartate (NMDA) receptor subunit and brain-derived neurotrophic factor (BDNF) mRNA, increased cholinergic innervation of the hippocampus and enhanced spatial learning and memory. A cross-fostering arm of the study, in which pups from neglectful mothers were raised by high-nurturing mothers, provided evidence for a direct relationship between maternal behaviour and hippocampal development. Interestingly, not all neonates were equally sensitive to variations in maternal care, illustrating the permanent intricate interrelationship between genes and environment.

Tissue changes in AD are well established. However, early diagnosis and detection of AD has been a challenging problem. Post-mortem studies had revealed that patients with AD have neurofibrillary tangles in the olfactory epithelium and entorhinal–hippocampal–subicular regions. Based on this, a study was conducted to assess whether olfactory impairment in individuals with mild cognitive impairment may predict subsequent AD [29]. In this study, tests of olfaction and olfaction-related anosognosia were both sensitive and specific in predicting subsequent illness. Interestingly, one of the

candidate genes for psychosis identified through a convergent functional genomics approach, G-protein-coupled receptor kinase 3 (GRK3) [5], is highly expressed in olfactory cortex, where it plays a role in odorant receptor desensitization [30]. This opens the interesting possibility that direct or indirect alterations in this gene's activity could lead to early, detectable phenotypical changes in a subset of psychotic disorders.

Mutations in the amyloid precursor protein (APP) and presenilin-1 and -2 genes (PS-1, PS-2) cause AD. Transgenic mice engineered to carry both mutant genes (PS/APP) develop AD-like deposits composed of β-amyloid (Aβ) at an early age. A study using these mice showed an inflammatory response occurring in response to the amyloidosis [31]. Both fibrillar and nonfibrillar Aβ (diffuse) deposits were visible in the frontal cortex by 3 months, and the amyloid load increased dramatically with age. The number of fibrillar Aβ deposits increased up to the oldest age studied (2.5 years old), whereas there were less marked changes in the number of diffuse deposits in mice over 1 year old. Activated microglia and astrocytes increased synchronously with amyloid burden and were, in general, closely associated with deposits. Cyclooxygenase-2, an inflammatory response molecule involved in the prostaglandin pathway, was up-regulated in astrocytes associated with some fibrillar deposits. Complement component 1q, an immune response component, strongly co-localized with fibrillar Aβ, but was also up-regulated in some plaque-associated microglia. These results, showing that cyclooxygenase-2 and complement component 1q levels increase in response to the formation of fibrillar Aβ in a transgenic model of AD, are of interest in view of human clinical epidemiological data suggesting that the use of nonsteroidal anti-inflammatory drugs (NSAIDs), among other things, down-regulates cyclooxygenase-2, delaying the onset and progression of the illness [32].

Peripheral Molecular Markers

Because, at present, the diagnosis of schizophrenia relies principally on descriptive behavioural and symptomatic information, the identification of a peripheral measurable marker might enable simpler, more rapid and more accurate diagnosis and monitoring. Human peripheral blood lymphocytes have been found to express several dopamine receptors (D3, D4 and D5) by molecular biology techniques and binding assays. It has been speculated that these dopamine receptors found on lymphocytes may reflect receptors found in the brain. The D3 dopamine receptor on lymphocytes has been demonstrated to correlate with schizophrenia [33]. In that study there was a significant elevation (at least two-fold) in the mRNA level of the D3 but not of the D4 dopamine receptor in schizophrenic patients. This increase was not affected by different antipsychotic drug treatments (typical

or atypical). Patients not receiving medication exhibited the same pattern, indicating that this change is not a result of medical treatment. The authors propose that the D3 receptor mRNA in blood lymphocytes could constitute a trait marker for identification and follow-up of schizophrenia.

A similar study from another group confirmed findings of changes of dopamine receptor mRNA levels in schizophrenic patients [34]. Forty-four schizophrenics who had been receiving drug medication for more than 3 years, 28 schizophrenics who had been drug-free for more than 3 months, 15 drug-naive schizophrenic patients, and 31 healthy persons were enrolled. Quantitative PCR of the mRNA was used to investigate the expression of D3 and D5 dopamine receptors in peripheral lymphocytes. The gene expression of dopamine receptors was compared in each group. In drug-free and drug-naive patients, the dopamine receptors of peripheral lymphocytes were sequentially studied in the second and eighth weeks after administration of antipsychotic medication. In the drug-free schizophrenic group, the D3 dopamine receptor mRNA expression of peripheral lymphocytes was significantly increased compared to that in controls and the drug-medicated schizophrenics, and D5 dopamine receptor mRNA expression was increased compared to that in the drug-medicated schizophrenics. Interestingly, the group of patients with increased dopamine receptor expression had more severe psychiatric symptoms. These results seem to suggest that the molecular biologically determined dopamine receptors of peripheral lymphocytes are reactive in response to antipsychotic treatment, and that increased expression of dopamine receptors in peripheral lymphocytes has possible clinical significance for subgrouping of schizophrenic patients.

Heterotrimeric G proteins play a pivotal role in post-receptor information transduction in cells and have been implicated in the pathophysiology and treatment of mood disorders. Changes have also been detected in G protein levels in post-mortem brains of patients with schizophrenia, where they could reflect an underlying abnormality or be an effect of antipsychotic treatment. A study aiming to eliminate this confound looked at receptor-coupled G proteins in mononuclear leukocytes obtained from 23 untreated patients with schizophrenia and 30 healthy controls [35]. Dopamine-enhanced guanine nucleotide binding capacity to G_s protein through D1/D5 receptors in mononuclear leukocytes of untreated patients with schizophrenia was significantly increased in comparison with that in healthy subjects, and positively correlated with both the total Positive and Negative Syndrome Scale (PANSS) score and the positive subscale. β-adrenergic and muscarinic receptor-coupled G protein functions, as well as $G_{s\alpha}$, $G_{i\alpha}$ and G_β immunoreactivities, were similar to those in healthy subjects. The lack of relationship to drug treatment makes these findings of elevated dopamine receptor-coupled G_s protein measures in mononuclear leukocytes of patients with schizophrenia useful as potential trait diagnostic markers.

GRK3, a candidate gene for mania and psychosis identified through a convergent functional approach [5], has been implicated in dampening signal transduction from G-protein-coupled receptors in neuronal and other tissues, and is present in human lymphocytes. There are some preliminary data indicating alterations of its levels in lymphocytes from patients with bipolar disorders [5], and it may deserve further scrutiny in subtypes of schizophrenia also, as a possible peripheral marker.

Advances and Insights from Convergent Functional Genomic Studies

A recently published study [18] looked at gene expression changes in the prefrontal cortex (PFC), an area implicated in schizophrenia by imaging studies in post-mortem brains of matched pairs of schizophrenics and control subjects. It identified a number of genes involved in presynaptic function as being abnormally decreased, the most strongly implicated being *N*-ethylmaleamide sensitive factor and synapsin II. The data suggest that subjects with schizophrenia may share a common abnormality in presynaptic function.

Advances and Insights from Pharmacological Studies

An interesting emerging direction is the study of the use of potential neuroprotective agents. Damage from free radicals and oxidative stress has been proposed as a cause of tardive dyskinesia. A recent study in rats [36] investigated whether neuroleptic medications may affect the motor system through the creation of free radicals, and whether structural brain changes related to oxidative damage may disrupt normal striatal function. The study showed that rats treated chronically with fluphenazine had significantly lower striatal cholinergic neuron densities than those that did not receive antioxidants. Rats exposed to a diet consisting of antioxidants had significantly higher neuron densities in each of the three regions tested than did those that did not receive antioxidants. Clinical trials of the antioxidant vitamin E for reducing the severity of symptoms of tardive dyskinesia have had mixed results [37], but it may be that irreversible neuronal loss had already occurred and that antioxidant supplementation should be instituted very early on during neuroleptic and other treatments that may lead to oxidative damage, in order to have an impact.

Lithium has recently been added to the list of potential neuroprotective agents. Rodent studies have shown that lithium exerts neurotrophic or neuroprotective effects [6]. An imaging study in patients [38] used three-dimensional magnetic resonance imaging and brain segmentation to study

grey-matter volume with chronic lithium use in patients with bipolar mood disorder. Grey-matter volume increased after 4 weeks of treatment, probably because of neurotrophic effects. This has led the authors to propose that low-dose lithium may have a potential use as a prophylactic agent for age-related neuronal loss and dementia [39].

The role of nicotine in improving cognition [40] may explain the high incidence of smoking in schizophrenics as a form of self-medication. Pharmacological, clinical and epidemiological data also support a role for nicotine in delaying the onset and perhaps slowing down the progression of AD. Interestingly, nicotine produces a long-lasting elevation of nerve growth factor (NGF) production when administered experimentally directly to the hippocampus of rats. In the central nervous system (CNS), NGF has powerful effects on the cholinergic system. It promotes cholinergic neuron survival after experimental injury as well as maintaining and regulating the phenotype of uninjured cholinergic neurons. In addition to these neurotrophic effects mediated by gene expression, NGF has a rapid neurotransmitter-like action to regulate cholinergic neurotransmission and neuronal excitability. Consistent with its actions on the cholinergic system, NGF can enhance function in animals with cholinergic lesions and has been suggested as being to be useful in humans with AD [16, 17]. However, the problems of CNS delivery, and of potential side effects such as pain, limit the clinical efficacy of NGF. Drug treatment strategies to enhance production of NGF in the CNS may be useful in the treatment of AD. Nicotine may be one such agent [41].

MOOD DISORDERS

Clinical Comorbidity Underlined by Overlapping Biological Mechanisms

We will consider in this section bipolar disorders and depression. Anxiety disorders, often closely related and comorbid, will be considered in the next section. Three emerging themes in the biology of mood disorders are:

1. Mood disorders, even what was considered unipolar depression, involve cycling and can broadly be viewed as part of a bipolar spectrum, or spectra [42].
2. There is extensive comorbidity with other mental disorders; it is the rule rather than the exception.
3. There is a longitudinal organic progression in the lifetime history of mood disorder, with progressive end-organ changes and damage.

Tissue Remodelling

Increasing evidence suggests that mood disorders are associated with a reduction in regional CNS volume and neuronal and glial cell atrophy or loss. Catecholamines have been recently implicated in both neurotoxic and neuroprotective phenomena [43, 44], and lithium has been demonstrated to increase robustly the levels of the cytoprotective, anti-apoptotic B-cell lymphoma protein-2 (bcl-2) in both cultured cells and areas of rodent brain [6]. Therefore, tissue remodelling may be relevant as a mechanistic substrate underlying the behavioural phenotypes and long-term effects of drugs, as well as the progressive nature of mood disorders, which may best be understood as an end-organ damage paradigm.

The influence of stress and glucocorticoids on neuronal pathology has been demonstrated in animal and clinical studies. It has been proposed that stress-induced changes in the hippocampus may be central to the development of depression in genetically vulnerable individuals. A study investigating the effect of antidepressants on hippocampal neurogenesis in the adult rat [45] used the thymidine analogue bromodeoxyuridine (BrdU) as a marker for dividing cells. The study demonstrated that chronic antidepressant treatment significantly increased the number of BrdU-labelled cells in the dentate gyrus and hilus of the hippocampus. Administration of several different classes of antidepressant, but not non-antidepressant, agents was found to increase BrdU-labelled cell number, indicating that this was a common and selective action of antidepressants. In addition, up-regulation of the number of BrdU-labelled cells was observed after chronic, but not acute, treatment, consistent with the time course for the therapeutic action of antidepressants. Additional studies demonstrated that antidepressant treatment increased the proliferation of hippocampal cells and that these new cells matured and become neurons, as determined by triple labelling for BrdU and neuronal- or glial-specific markers. These findings raise the interesting possibility that increased cell proliferation and increased neuronal number may be a mechanism by which antidepressant treatment overcomes the stress-induced atrophy and loss of hippocampal neurons and may contribute to the therapeutic actions of antidepressant treatment. Their likely trophic actions on other areas of the brain merit further exploration.

Additional evidence implicates the PFC in addition to the hippocampus as a site of neuropathology in depression. The PFC may be involved in stress-mediated neurotoxicity because stress alters PFC functions and glucocorticoid receptors, the PFC is directly interconnected with the hippocampus, and metabolic alterations are present in the PFC in depressed patients. Post-mortem studies in major depression and bipolar disorders provide evidence for specific neuronal and glial histopathology in mood disorders [8]. Three patterns of morphometric cellular changes were observed in that

study: cell loss (subgenual PFC), cell atrophy (dorsolateral PFC and orbitofrontal cortex) and increased numbers of cells (hypothalamus, dorsal raphe nucleus). The study suggests that cellular changes in mood disorders may be due to stress and prolonged PFC remodelling, with a role played by neurotrophic/neuroprotective factors. Furthermore, the precise anatomic localization of dysfunctional neurons and glia in mood disorders may lead to specific cortical targets at molecular and cellular level for the development of novel antidepressants and mood stabilizers.

Physical activity may also impact mood through neurotrophic effects on the brain. In mice, running was shown to increase neurogenesis in the dentate gyrus of the hippocampus, a brain structure that is important for memory function [46]. Additionally, in that study, spatial learning and long-term potentiation (LTP) were tested in groups of mice housed either with a running wheel (runners) or under standard conditions (controls). Mice were injected with BrdU to label dividing cells and trained in the Morris water maze. LTP was studied in the dentate gyrus and area CA1 in hippocampal slices from these mice. Running improved water maze performance, increased BrdU-positive cell numbers, and selectively enhanced dentate gyrus LTP. The results suggest that physical activity can regulate neurogenesis, synaptic plasticity and learning.

There are clinical observations that are possible correlates for these effects. Several reports indicate that physical activity can reduce the severity of symptoms in depressed patients. Some data suggest that even a single exercise bout may result in a substantial mood improvement. A study evaluating the short-term effects of a 10-day training programme on patients with moderate to severe major depression [47] found clinically relevant and statistically significant reduction in depression scores, suggesting that aerobic exercise can produce substantial improvement in mood in patients with major depressive disorders in a short time. Another study compared physical exercise with standard drug treatment for depression [48]. The study assessed the status of 156 adult volunteers with major depressive disorder (MDD) 6 months after completion of a study in which they were randomly assigned to a 4-month course of aerobic exercise, sertraline therapy, or a combination of exercise and sertraline. After 4 months, patients in all three groups exhibited significant improvement; the proportion of participants with remission was comparable across the three treatment conditions. After 10 months, however, subjects in remission in the exercise group had significantly lower relapse rates ($p < 0.01$) than subjects in the medication group. Exercising on one's own during the follow-up period was associated with a reduced probability of depression diagnosis at the end of that period. Obviously, exercise has a variety of physiological and endocrinological effects on the body, but an intriguing possibility exists that neurotrophic effects may underlie some of the positive effects of exercise on mood.

Endocrinological Aspects

Several studies have underlined the high prevalence of psychiatric symptoms and disorders in endocrine diseases. More recently, the role of sex hormones in the differential spectrum of mood disorders in women versus men [42] and the role of hormone replacement therapy as an adjuvant treatment in mood disorders [49–51] are receiving increasing recognition and attention. The underlying biology may be related to the integration and cross-talk of signal-processing cascades from membrane-bound neurotransmitter receptors with those from nuclear ligand-activated transcription factors such as hormone receptors for oestrogen, progesterone and testosterone.

Estradiol is known to affect a number of neurotransmitter systems in the brain. Stress and corticotropin-releasing hormone inhibit the reproductive axis. A study examining whether reproductive axis hormone secretion is inhibited in women with depression, similar to what has been observed to be caused by stress in numerous species, found that the blood levels of reproductive hormones were mostly normal in women with depression, but the blood level of estradiol was significantly lower [52].

Decreased *growth hormone* (GH) response to pharmacological stimulation has been found in children and adolescents during an episode of major depressive disorder and after recovery. GH secretion is similarly altered in children and adolescents who had never experienced depression but were at high risk of developing depression [53]. These results suggest that the decreased GH response found in high-risk subjects may represent a trait marker for depression in children and adolescents. It is interesting to note that one of the candidate psychogenes identified by our work using convergent functional genomics [5] described below is insulin-like growth factor 1 (IGF1), a downstream effector in the GH pathway.

Thyroid disorders also strongly affect mood. A study to evaluate the prevalence of mental disorders in 93 inpatients affected by different thyroid diseases during their lifetimes, by means of standardized instruments, showed higher rates of panic disorder, simple phobia, obsessive-compulsive disorder, MDD, bipolar disorder and cyclothymia in thyroid patients than in the general population [54]. These findings may suggest either that thyroid abnormalities effect secondary mood changes, or that the co-occurrence of mental and thyroid diseases may be the result of common biochemical abnormalities.

Lithium is known to interact with the thyroid axis and causes hypothyroidism in a subgroup of patients, which compromises its mood-stabilizing effects. Lithium was reported to alter thyroid hormone metabolism in the rat brain, and a study investigating whether these effects were mediated through regulation of thyroid hormone receptor (THR) gene expression found that chronic lithium treatment appeared to regulate THR gene expression in a subtype- (isoform) and region-specific manner in the rat brain [55]. This study raises the possibility that the observed effects of lithium on THR gene expression may be related to its therapeutic efficacy in the treatment of bipolar disorders.

Advances and Insights from Convergent Functional Genomic Studies

The initial description and application of the concept of convergent functional genomics was in identifying genes involved in mania and psychotic mania [5]. Methamphetamine treatment of rats as an animal model for psychotic mania was used. Specific brain regions—PFC, amygdala—were analysed comprehensively for changes in gene expression using oligonucleotide GeneChip microarrays. These regions had been implicated in mood and psychotic disorders by previous studies in animal models and imaging studies in humans. The data were cross-matched against human genomic loci associated with either bipolar disorder or schizophrenia, which had been previously identified by human genetic linkage studies. Using this convergent approach, we have identified several novel possible candidate genes that may be involved in the pathogenesis of mood disorders and psychosis—signal transduction molecules like GRK3, transcription factors like the clock gene D-box binding protein (DBP), growth factors such as IGF1, metabolic enzymes like farnesyl-diphosphate farnesyltransferase 1 (FDFT1) involved in cholesterol biosynthesis and sulfotransferase 1A1 (SULT1A1) involved in dopamine metabolism, and others. Furthermore, for one of these genes, GRK3, preliminary experiments by Western blot analysis found evidence for decreased protein levels in a subset of patient lymphoblastoid cell lines that correlated with disease severity.

We also proposed a novel paradigm for classification of these and other candidate genes involved in mental disorders, by analogy to cancer biology, into two prototypical categories, psychogenes and psychosis-suppressor genes. Genes whose activity promotes processes that lead to mania or psychosis could be called "psychogenes", by analogy to oncogenes. Conversely, genes whose activity suppresses processes that lead to these mental disorders could be called "psychosis-suppressor genes". This classification, while probably oversimplistic, may have heuristic value for psychiatry as it has had for cancer biology, by providing a framework for understanding the roles of putative disease genes in pathophysiology and as targets for developing treatment strategies. Using this paradigm, and on the basis of their biology, DBP, IGF1 and FDFT1 were classified as candidate psychogenes, and GRK3 and SULT1A1 were classified as candidate psychosis-suppressor genes [5].

Furthermore, it is possible that genes that show concomitant changes in expression levels in such studies may be interacting pathophysiologically, and warrant further analysis as co-acting gene groups. The concept that

"genes that change together act together" provides a straightforward testable model for unravelling complex polygenic diseases like bipolar disorders, schizophrenia and others, including non-mental disorders.

Advances and Insights from Pharmacological Studies

An interesting study supporting the concept of progressive end-organ changes was reported recently [56]. The authors investigated the relationship between the number of lifetime episodes of affective disorder and the antimanic response to lithium, divalproex or placebo. An apparent transition in the relationship between number of previous episodes and response to antimanic medication occurred at about 10 previous episodes. For patients who had experienced more episodes than this, the response to lithium resembled the response to placebo but was worse than the response to divalproex. For patients who had experienced fewer episodes, however, the responses to lithium and divalproex did not differ and were better than the response to placebo. This differential response pattern was not related to rapid cycling or mixed states. The authors conclude that a history of many previous episodes was associated with poor response to lithium or placebo but not to divalproex.

Medications have potential side effects, which may reduce patient compliance. Some patients also have a psychological resistance to being on psychotropic medications long-term, hence their seeking other approaches, including "alternative medicine". One such approach for which there are actually good epidemiological, clinical and biological data is using supplementation with omega-3 fatty acids, which are long-chain, polyunsaturated fatty acids that are a normal component of cell membranes. They are found in the diet in enriched form in plant and marine sources, such as fish oil. Unlike saturated fats, which may have negative health consequences, omega-3 fatty acids have been associated with health benefits in cardiovascular disorders and arthritis.

Omega-3 fatty acids have been proposed to be potentially efficacious in a number of mental disorders [57–59]. Diminished levels of omega-3 fatty acids have been reported in mood disorders like depression. An epidemiological study looking at fish consumption and depressive symptoms in the general population in Finland found that the likelihood of having depressive symptoms was significantly higher among infrequent fish consumers than among frequent fish consumers, even after adjusting for potential confounders [60]. One double-blind placebo-controlled trial reported favourable results using omega-3 fatty acids as an adjunctive treatment in bipolar disorder [61]. Their molecular mechanism of action is still being elucidated, but, like the case in mood stabilizers, it seems to have to do with impacting on cell membrane function and signal transduction from the membrane to the nucleus through protein kinase signalling [62].

ANXIETY DISORDERS

Clinical Comorbidity Underlined by Overlapping Biological Mechanisms

From an evolutionary standpoint, anxiety is probably a signal of alarm to the organism in uncertain and potentially dangerous situations. While pure, or, more precisely, *mainly* anxiety disorders may exist, there is an emerging recognition that there are significant interactions and impact with both mood and cognition. In terms of interactions with mood, anxiety and low mood may translate as fear, whereas anxiety and high mood may translate as irritability and anger. In terms of interactions with cognition, high anxiety may be a component of paranoid ideation, and lack of anxiety a component of antisocial psychotic acts.

Tissue Remodelling

Preclinical studies demonstrate that early anxiety and stress can alter the development of the hypothalamic–pituitary–adrenal (HPA) axis, hypothalamic and extrahypothalamic corticotropin-releasing hormone (CRH), monoaminergic and γ-aminobutyric acid/benzodiazepine systems. Stress has also been shown to promote structural and functional alterations in brain regions similar to those seen in adults with chronic anxiety and depression [63].

As mentioned, stress has been shown to lead over time to cell death in the hippocampus. Elevated glucocorticoid levels produce hippocampal dysfunction and correlate with individual deficits in spatial learning in aged rats. Aged humans with significant prolonged cortisol elevations showed reduced hippocampal volume and deficits in hippocampus-dependent memory tasks compared to normal-cortisol controls [64]. Moreover, the degree of hippocampal atrophy correlated strongly with both the degree of cortisol elevation over time and current basal cortisol levels in the studied population. Therefore, basal cortisol elevation may cause hippocampal damage and impair hippocampus-dependent learning and memory in humans.

Endocrinological Aspects

CRH is a critical coordinator of the HPA axis. In response to stress, CRH released from the paraventricular nucleus (PVN) of the hypothalamus activates CRH receptors on anterior pituitary corticotropes, resulting in release of adrenocorticotropic hormone (ACTH) into the bloodstream. ACTH in turn activates ACTH receptors in the adrenal cortex to increase synthesis

"genes that change together act together" provides a straightforward testable model for unravelling complex polygenic diseases like bipolar disorders, schizophrenia and others, including non-mental disorders.

Advances and Insights from Pharmacological Studies

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dopaminergic neurotransmission, illustrates this point [72]. Dopaminergic neurons exert a major modulatory effect on the forebrain. DARPP-32, which is enriched in all neurons that receive a dopaminergic input, is converted in response to dopamine into a potent protein phosphatase inhibitor. Mice generated to contain a targeted disruption of the DARPP-32 gene showed profound deficits in their molecular, electrophysiological and behavioural responses to dopamine, drugs of abuse and antipsychotic medication.

Further illustrating the intimate interplay between cognition and addiction, dopamine mediated mechanisms of addiction are also likely to involve some of the molecular mechanisms of memory formation [73].

Tissue Remodelling

Another emerging theme is that of progressive brain changes and end-organ damage resulting from sustained abuse of drugs. Behavioural abnormalities associated with addiction are very long-lived. It is being increasingly appreciated that chronic drug exposure causes stable changes in the brain at the molecular and cellular levels that underlie these behavioural abnormalities [74].

A recent study with methamphetamine users illustrates this point. While illicit stimulants are often used to enhance attention and alertness and generally speed up the thought process, chronic users had a dose-dependent decrease of performance in neuropsychological tests that assess recall, ability to manipulate information, ability to ignore irrelevant information, and abstract thinking [75]. A positron tomography study in methamphetamine abusers revealed an association of dopamine transporter reduction with psychomotor impairment [76]. A parallel study from the same group of investigators found higher cortical and lower subcortical metabolism in detoxified methamphetamine abusers [77]. These results suggest that, in humans, methamphetamine abuse results in lasting changes in the function of dopamine- and non-dopamine-innervated brain regions.

One such molecular switch underlying long-term neural plasticity is DeltaFosB, a transcription factor that has been implicated in drug addiction and movement disorders [78].

Advances and Insights from Convergent Functional Genomic Studies

Acute methamphetamine administration in rats has been used as an animal model of mania [5]. The candidate genes identified in that study through a convergent functional genomics approach, as discussed in the section on

mood disorders, may also represent players involved in stimulant addiction and provide a mechanistic basis for comorbidity between bipolar disorders and stimulant abuse and addiction.

Cocaine enhances dopamine-mediated neurotransmission by blocking dopamine re-uptake at axon terminals. The striatum is one such site of action. Chronic exposure to cocaine up-regulates several transcription factors that alter gene expression and which could mediate the long-term neural and behavioural changes induced by the drug. One such transcription factor is DeltaFosB, a protein that persists in striatum long after the end of cocaine exposure. Using DNA microarray analysis of striatal tissue from both inducible transgenic mice engineered to overexpress DeltaFosB and mice treated with cocaine, cyclin-dependent kinase 5 (Cdk5) was identified as a downstream target gene of DeltaFosB [79]. Overexpression of DeltaFosB, or chronic cocaine administration, raised levels of Cdk5 messenger RNA, protein and activity in the striatum. Interestingly, injection of Cdk5 inhibitors into the striatum potentiated behavioural effects of repeated cocaine administration. This elegant study implicates the neuronal protein Cdk5 as a regulator of the effects of chronic exposure to cocaine, and identifies a novel cellular pathway as a potential target for pharmaceutical drug development.

Changes in brain gene expression are thought to be responsible for the tolerance, dependence and neurotoxicity produced by chronic alcohol abuse. DNA microarrays have been used recently with some success in studies of alcoholism [20]. RNA was extracted from post-mortem samples of superior frontal cortex of alcoholics and non-alcoholics. Relative levels of RNA were determined by array techniques. Expression levels were determined for over 4000 genes, and 163 of these were found to differ by 40% or more between alcoholics and non-alcoholics. Analysis of these changes revealed a selective reprogramming of gene expression in this brain region, particularly for myelin-related genes, which were down-regulated in the alcoholic samples. In addition, cell cycle genes and several neuronal genes were changed in expression. The investigators conclude that the observed gene expression changes suggest a mechanism for the loss of cerebral white matter in alcoholics as well as alterations that may lead to the neurotoxic actions of ethanol.

A recent study comprehensively catalogued gene expression changes in rat brains following acute and chronic exposure to δ-9-tetrahydrocannabinol (THC), the active ingredient in marijuana, using microarray technology profiling a total of 24 456 cDNAs [80]. Of these, only 49 different genes showed specific changes in expression compared to control animals, including some signal transduction molecules (prostaglandin D synthase, calmodulin), and structural molecules [neural cell adhesion molecule (NCAM), myelin basic protein].

The sequencing of the human and other mammalian genomes is a watershed event that will help us to understand the biology of addiction by enabling us to identify genes that contribute to individual risk for addiction

and those through which drugs cause addiction. A preliminary search of a draft sequence of the human genome for genes related to desensitization of receptors that mediate the actions of drugs of abuse on the nervous system yielded multiple potential candidates and illustrates the impact this methodology can make in speeding up the discovery process [70].

Advances and Insights from Pharmacological Studies

Ondansetron, an anti-nausea drug best known for its use in cancer chemotherapy, has been reported to be effective in reducing drinking, especially in patients with early-onset alcoholism (before age 25) [81]. In their discussion, the authors speculate that ondansetron changes the balance of activity among the neurotransmitters dopamine and serotonin. In particular, it reduces the activity at one of the serotonin receptors, 5-HT3; in previous animal studies, blocking this receptor had been found to reduce the consumption of alcohol. It is hypothesized that early-onset alcoholics may carry a genetic variant of the receptor that makes them more vulnerable to the addictive effects of alcohol. Interestingly, the blood test used to measure alcohol use in this study is a new one: it measures carbohydrate-deficient transferrin (CDT), which accumulates in the blood with sustained heavy drinking, as haemoglobin A_{1c} does in diabetes, and persists at elevated levels for weeks after drinking stops. The test was recently approved by the US Food and Drug Administration for use in alcohol treatment centres and may soon become widespread.

SLEEP DISORDERS

Clinical Comorbidity Underlined by Overlapping Biological Mechanisms

Sleep is altered in a variety of mental disorders, both as a consequence of the illness and secondary to pharmacological treatment. Mania and anxiety disorders can lead to sleep decrease, whereas anergic depression and negative-symptoms schizophrenia can lead to increases in the duration of sleep. As one of the few objective parameters to be investigated during a psychiatric interview, sleep may be viewed as the "temperature" of the psychiatric clinical exam. The extreme phenotype of narcolepsy has led to the identification of a family of brain peptides called hypocretins as involved in sleep regulation. Another promising line of research is represented by clock genes. Clock genes regulate circadian rhythms, are highly conserved from plants to man [82] and may be molecular substrates mediating the interface of sleep and psychiatric syndromes.

Tissue Remodelling

Narcolepsy is a disorder characterized by sleep attacks, cataplexy, disrupted sleep patterns and hypnogogic hallucinations. Neurons containing the neuropeptide hypocretin (orexin), identified using a combination of animal models and genetics as described below, are located exclusively in the lateral hypothalamus and send axons to numerous regions throughout the CNS, including the major nuclei implicated in sleep regulation. In a post-mortem study of human brains [83], investigators have found that narcoleptics had 85–95% fewer hypocretin-expressing neurons in their hypothalamus than did matched controls. This suggests that a neurodegenerative process may have affected the hypocretin neurons.

Advances and Insights from Convergent Functional Genomic Studies

Two animal models of narcolepsy that have been instrumental in understanding the pathophysiology of the disease are the canarc-1 mutant dogs, which have a spontaneous mutation, and the orexin knockout mice, where hypocretin/orexin was deleted by a purposeful experimental transgenic approach.

Positional cloning was used to identify an autosomal recessive mutation responsible for narcolepsy in the canine model [84]. The study determined that canine narcolepsy is caused by disruption of the hypocretin (orexin) receptor 2 gene (Hcrtr2).

Assessed using behavioural and electroencephalographic criteria, orexin knockout mice [85] exhibit a phenotype strikingly similar to that of human narcolepsy patients, as well as to canarc-1 mutant dogs, the only known monogenic model of narcolepsy. Modafinil, an anti-narcoleptic drug with ill-defined mechanisms of action, was observed in those mice to activate orexin-containing neurons.

These results identify hypocretins as major sleep-modulating neurotransmitters and open novel potential therapeutic approaches for narcoleptic patients.

Familial advanced sleep phase syndrome (FASPS) is an autosomal dominant circadian rhythm variant; affected individuals are "morning larks" with a 4-hour advance of the sleep, temperature and melatonin rhythms. Human genetic linkage studies localized the FASPS gene near the telomere of chromosome 2q. A strong candidate gene (hPER2), a human homologue of the period gene in *Drosophila*, maps to the same locus. Affected individuals were shown to have a serine-to-glycine mutation within the casein kinase Iε (CKIε) binding region of hPER2, which causes hypophosphorylation by CKIε in vitro [86]. A stable alteration in human sleep behaviour can

thus be attributed to a missense mutation in a clock component, hPER2, which alters the circadian period.

Another clock gene, D-box binding protein (DBP), was identified as a candidate for being involved in mood and psychotic disorders by a convergent functional genomics approach [5], as described earlier in this chapter. DBP is a transcriptional activator that shows a robust circadian rhythm. DBP knockout mice show a reduced amplitude of circadian modulation of sleep time, a reduction in the consolidation of sleep episodes and reduced locomotor activity, a picture that is not unlike depression [87]. Clock genes have been shown to be important for the development of behavioural sensitization to repeated stimulant exposure [88]. Taken together, the converging lines of evidence about connections between clock genes, stimulant sensitization, circadian rhythmicity, sleep and mood disorders make DBP an interesting target for further studies of the bidirectional interface between disorders of sleep and other psychiatric disorders.

Advances and Insights from Pharmacological Studies

The interplay between sleep disturbances and mood disorder is underlined, for example, by the use of stimulants to augment antidepressant treatment in patients who have had only a partial response to first-line therapy. Modafinil is a novel psychostimulant that has shown efficacy in, and is marketed for, treating excessive daytime sleepiness associated with narcolepsy. The mechanism of action of modafinil is unknown, but, unlike other stimulants, the drug is highly selective for the CNS, has little effect on dopaminergic activity in the striatum, and appears to have a lower abuse potential. In a retrospective case series of seven patients with DSM-IV depression (four with major depression and three with bipolar depression) in whom modafinil was used to augment a partial or non-response to an antidepressant, all patients achieved full or partial remission within 1–2 weeks [89]. These preliminary results suggest that modafinil may be of use as an augmenter of antidepressants, especially in patients with residual tiredness or fatigue. It may also be an interesting choice in treating negative symptoms associated with schizophrenia, although rigorous controlled studies need to be carried out.

CONCLUSIONS AND FUTURE DIRECTIONS

Increasing Confluence of Psychiatry and Neurology

As psychiatry better understands the underlying structural and molecular changes associated with mental disorders, and as neurology explores

further the behavioural, cognitive and affective aspects of neurological disorders of the brain, there is increasing overlap between the two specialties that may lead down the road to a unified specialty, brainology [13].

Blurring of the Separation Between Axis I and Axis II

The constantly increasing psychopharmacological armamentarium at our disposal is significantly impacting axis II disorders, and has revealed the separation between axis I and axis II to be somewhat artificial. An example of this is that personality disorder scores improve with effective pharmacotherapy of depression [90]. Molecular genetic research will also benefit from viewing axis I and axis II as lying on a continuum-of-severity spectrum, together with even softer forms that are currently classified as temperaments. Illustrating this trend is a recent study identifying the association between a polymorphism in the promoter region of the human dopamine receptor D4 (DRD4) gene and novelty-seeking personality traits [91].

Better Phenotypical Definitions

Psychiatry needs better, more precise quantitative descriptions of mental phenomena and the phenotypes of different disorders in order to improve patient care and speed up the convergent functional genomics and pharmacological discovery processes. This has not kept pace with the contemporary progress in molecular genetics and brain imaging, and may provide a rate-limiting step for future progress [42]. Our expectation is that the constant interplay between molecular and cellular biology, imaging and clinical research will avoid this roadblock.

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