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Levels of analysis in psychiatric research

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Abstract

Most of the major psychiatric disorders have been analyzed at each of several different levels. For example, at the broadest level, epidemiological studies have served to establish the incidence of disorders like schizophrenia and major depression in a number of different populations. Family and twin studies have been important in determining the heritability of certain mental illnesses, and chromosomal and linkage analyses have identified a number of discrete loci that appear to be implicated in disease susceptibility or, even directly, in the pathogenesis of some disorders. In a few cases, specific genes have been found to be mutated or polymorphic and proteins they encode are currently being analyzed. This article reviews how these different levels contribute to our understanding of a number of psychiatric disorders, including drug addiction, which has been the focus of much of our own work.

The recognition that psychiatric illnesses are disorders of the brain has opened new avenues for the analysis of their etiology and pathogenesis, as this Special Issue of *Development and Psychopathology* attests. Given the contentious history of psychiatry, however, this simple statement might still arouse a certain level of consternation. It is still unfortunately the case that, for some in psychiatry, "brain" remains a code word for "genetic" or "biological" determinism. In fact, modern neuroscience is focused not only on genes, cells, and synapses but also on "top down causality" mediated by experience. The brain is an exceedingly complex structure that is given its initial form by gene–environment interactions and remodeled over the life span by ongoing gene–environment interactions initiated by experience, drugs, and other environmental influences. Although scientists commonly utilize reductionist strategies to solve particular problems, the ultimate goal of neuroscience in psychiatry is not to reduce behavior to molecular processes. To understand the problems of psychiatry, explanations are required at multiple levels of analysis from the population level (i.e., epidemiology); through individual and group behavior, neuropathology, and systems-level neurobiology, to cellular and molecular neurobiology. It is becoming increasingly possible to consider the etiology of at least some mental illnesses at several of these levels. Leaving aside considerations of such philosophical issues as the nature of the mind and its relation to the brain, we consider the following seven levels of analysis: epidemiological; neuropathological; chromosomal; family, twin, and adoption studies; genetic linkage analysis; specific genes; and proteins and protein interactions.

It is still early in the history of this science: as yet, only a few mental disorders have been amenable to analysis at every level. We have accordingly chosen to select those examples that best exemplify the use of the currently available analytical tools and methods and to briefly describe what light they have thrown on the relevant disorders. Given the limitations of space, we only touch on the clinical features of the disorders discussed and then only insofar as they bear on the level of analysis in question. In a subsequent section, we show how drug addiction, which has been the

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subject of much of our own work, has been (and continues to be) analyzed at several different levels and indicate the directions from which further progress in our understanding of the nature of addictive disorders is likely to come.

Nosology or the Question of Disease Phenotype

Although different levels of analysis are often treated as though they were independent of each other, such analyses must ultimately converge and lead to a congruent understanding. The substantial problems that exist in the classifications of mental disorders exemplify the difficulties that arise when information at different levels does not converge. This lack of convergence suggests that our understanding of the causes and pathogenesis of mental disorders is still in its early stages. The current approach to psychiatric diagnosis was largely shaped by Robins and Guze (1970), who argued that reliable and valid diagnoses could only emerge from careful clinical description of syndromes, family and laboratory studies, and the clear delineation of one disorder from another. The validation of putative diagnostic entities would then be possible from family studies and from long-term follow-up studies to establish the stability and natural history of the diagnosis. Research over the last 30 years, however, has shown that, to a significant degree, psychiatric diagnosis formulated by the very reasonable approach of Robins and Guze does not actually converge on unequivocal diagnoses. For example, if one were to focus on the stability of diagnosis, then only those individuals who have had a chronic illness would warrant the diagnosis of schizophrenia; however, such chronicity does not necessarily segregate with psychosis in families. Indeed, many clinically significant symptom clusters do not breed true. To cite another example, psychosis and rapid cycling may not be inherited with core mood symptoms in bipolar disorder. Similarly, mood and anxiety disorders may not be entirely genetically distinct but may share genetic vulnerability. Such findings were difficult to rationalize before it was recognized that mental disorders, like many common human illnesses, are not "caused" by single genetic mutations but are instead genetically complex. It is widely believed (although as yet unproven) that variants at multiple genetic loci must interact with each other and with nongenetic factors to produce illness. Genes play a significant role in almost all the significant behavioral phenotypes that have been examined, but they are best considered as susceptibility factors rather than as direct causes of the phenotype. The fact that loci must interact to produce the risk of a given phenotype may help to explain why it is often difficult to define the boundaries between manic-depressive illness and schizophrenia, or between certain anxiety and mood disorders. The real strength of the *Diagnostic Statistical Manual* (DSM-IV) and the International Statistical Classification of Diseases criteria for mental disorders is that they have made it possible for different observers to agree on a diagnosis using the given criteria (with some notable exceptions, such as several of the DSM-IV personality disorders). However, reliability does not necessarily imply validity and there is little evidence that the current diagnostic criteria truly "carve nature at the joints."

Levels of Analysis for Examining Normal Behavior and Psychopathology

Epidemiology

Epidemiology has played a significant role in defining the boundaries of mental disorders by providing data on patterns of symptom clusters within populations. More recently it has established the substantial burden of mental disorders as a cause of disability and premature mortality. Such analyses have been of particular value to policymakers.

Among the first large-scale epidemiological studies using current approaches to diagnosis was the survey of selected mental illnesses in five large US cities that involved the screening of almost 20,000 individuals (Robins & Regier, 1991). This and a number of more recent studies in the United Kingdom, Australia, and Greece have established the

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magnitude of the problem posed by mental illnesses in developed countries. Combining prevalence data with estimates of disability and disease related mortality, the World Health Organization (WHO) has published data on the burden of various disorders, including mental illnesses. Based on the measure known as the DALY (i.e., the years of healthy life lost to disability or death; Michaud, Murray, & Bloom, 2001; Murray & Lopez, 1996), mental illnesses account for 5 of the 10 leading causes of disability in the United States and major depression currently ranks as the leading cause. According to the WHO, by the year 2020 unipolar major depression will be second only to ischemic heart disease among the leading causes of disability (Michaud et al., 2001).

Among the more interesting findings that have emerged from these epidemiological studies is that some disorders are similar in their prevalence worldwide. For example, schizophrenia has been found to affect about 1% of the population, regardless of geographical location or ethnic and cultural differences. On the other hand, certain disorders affect varying percentages in different populations. Thus, in Taipei the prevalence of major depression is said to be 10-fold lower than in cities such as Florence, Paris, and Beirut (Weissman, Bland, Canino, Faravelli, Greenwald, Hwu, Joyce, Karam, Lee, Lellouch, Lepine, Newman, Rubio–Stipec, Wells, Wickramaratne, Wittchen, & Yeh, 1996) and the suicide rate in China appears to be 3–5 times the world average. It is interesting that, unlike other countries for which data are available, the suicide rate among Chinese women is 40% higher than for Chinese men.

These findings are only as robust as the diagnostic criteria on which they are based and, although useful, cannot be considered definitive until better approaches to disease classification are developed. Furthermore, in one important respect psychiatric epidemiology differs from that in most other medical disciplines, even if one only considers disorders that are genetically complex and heterogeneous. In internal medicine, for example, disorders like diabetes and hypertension can be diagnosed by objective criteria (blood glucose levels and blood pressure, respectively), but psychiatric diagnoses lack such objective indicators. The lack of objective diagnostic criteria has been a major obstacle to progress at "lower" levels of analysis, including the identification of possible genetic loci involved in different mental illnesses and neuroimaging studies. Paradoxically, the development of more rigorous diagnostic criteria will depend on information drawn from these lower levels of analysis. As unsatisfying as it sounds, clarification of the phenotype and studies of etiology and pathogenesis are likely to progress in a circular and iterative fashion.

Brain pathology in mental illness

Progress in other fields of medical research has nearly always begun with the elucidation of the underlying pathogenesis of disorders. We need only mention how the discovery of reduced levels of dopamine in the striatum and the concomitant degeneration of neurons in the substantia nigra stimulated research on Parkinson disease (PD) and its treatment, or how the discovery of the genetic mutations responsible for the different forms of spinocerebellar ataxia (a family of single gene disorders) brought clarity to the diagnosis of disorders that had been complicated by the array of differing clinical signs and symptoms. Regrettably, psychiatry has lagged behind these other disciplines, in large part because the problems of understanding pathogenesis are so difficult. If we leave aside brain deformities associated with various forms of mental retardation, distinct pathological changes have been recognized in only two or three disorders.

The most striking pathological changes seen in a psychiatric disorder are those associated with *Alzheimer disease* (AD). These changes were first observed as early as 1905 by Alois Alzheimer in the brain of a 51-year-old woman who suffered from severe memory loss in the years preceding her death. In addition to being appreciably shrunken, her brain showed extensive deposits of amyloid plaques throughout most of the cerebral cortex, together with distinctive "neurofibrilliary tangles." Alzheimer identified these changes as

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the hallmarks of what he termed "presenile dementia." In the 1960s it became clear that the same changes are evident in "senile dementia," and it is now generally accepted that the earlier and late onset forms of the disorder are neuropathologically (and probably etiologically) identical. The same is true of the pathological changes seen in the brains of individuals with trisomy 21 (Down syndrome) who survive into their 30s or 40s; however, considerations of space preclude a discussion of the extensive work that has been done on the molecular basis of amyloid plaques and neurofibrilliary tangles.

No mental illness has attracted more attention from pathologists than *schizophrenia*. Indeed, as Plum remarked, schizophrenia has become "the graveyard of neuropathologists" (Plum, 1972). The extensive literature on the subtle and often dubious microscopic changes seen in the cerebral cortex and thalamus of individuals with schizophrenia was reviewed recently by Harrison, whose conclusion that "despite a hundred year's research the neuropathology of schizophrenia remains obscure" aptly summarizes the current state of our knowledge (Harrison, 1999).

Computer assisted tomographic and volumetric magnetic resonance imaging (MRI) studies have corroborated findings from earlier autopsy studies in showing that schizophrenia is commonly associated with enlargement of the lateral and third ventricles (Lawrie & Abukmeil, 1998) and significant reductions in cortical volume, particularly in the prefrontal and temporal areas (Nelson, Saykin, Flashman, & Riordan, 1998). Such volumetric changes have been identified on the first manifestation of illness, indicating that the underlying neuropathology precedes the behavioral disorder and is not a consequence of chronic illness or treatment. Microscopic analyses of cortical tissue suggest that the gross shrinkage that is observed is probably not due to a loss of neurons but rather to a loss of neuropil (dendrites, axons, axon terminals, and synapses) and this is borne out by neurochemical and ultrastructural studies. These findings are consonant with the view that schizophrenia is a neurodevelopmental disorder and with microscopic observations that the brains of schizophrenics exhibit ectopically located neurons that stain positively for the enzyme nicotinamide adenine dinucleotide phosphate diaphorase in the white matter immediately beneath the cortex of the frontal and temporal lobes (Jones, 1995). These neurons are thought to be remnants of the cortical subplate, a transient structure during cortical development, and their presence just beneath the cortex could reflect an early disorder in neuronal migration. Unfortunately, the evidence on this point is equivocal. Prospective MRI mapping of brain development in children indicates that in rare early onset cases of schizophrenia there is a dynamic and accelerated gray matter loss first in the parietal region, progressing anteriorly into the temporal lobe, and finally into the sensorimotor and dorsolateral prefrontal cortices and frontal eye fields (Thompson, Vidal, Giedd, Gochman, Blumenthal, Nicolson, Toga, & Rapoport, 2001).

Although there is some indication of changes in the distribution of neurotransmitter receptors in schizophrenia and other mental disorders, it is worth adding that the search for pathological changes in the brains of individuals affected with bipolar disorder, major depression, obsessive–compulsive disorder, and other illnesses has, as yet, proven unproductive.

Chromosomal abnormalities

The finding that there is a strong genetic component to most mental illnesses prompted a number of researchers to examine the chromosomes of individuals suffering from one or other disorder in the hope that identifiable and consistent changes might throw light on the locus of the genes involved. The success of this approach in the diagnosis of various neoplasias like Burkitt lymphoma and chronic myelogenous leukemia gave impetus to this effort.

In a sense this approach may be considered a refinement of the search for other pathological changes, and for this reason it is convenient to consider chromosomal abnormalities here. The characteristic chromosomal staining patterns seen in fluorescence and Giemsa

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preparations make the detection of chromosomal duplications, deletions, translocations, and other abnormalities relatively straightforward. Although there are many reports of chromosomal changes in the major psychoses and affective disorders (see, e.g., DeLisi, Devoto, Lofthouse, Poulter, Smith, Shields, Bass, Chen, Vita, Morganti, Ott, & Crow, 1994; DeLisi, Friedrich, Wahlstrom, Boccio–Smith, Forsman, Eklund, & Crow, 1994), very few have been confirmed and none seems to be directly implicated in the pathogenesis of these disorders (Baron, 2001). However, this approach has proved to be invaluable in the early diagnosis of a number of developmental disorders, including trisomy 21, Williams syndrome, and Fragile X mental retardation, which we shall consider briefly.

Trisomy 21 (Down syndrome) is the most common and readily identifiable of all chromosomal abnormalities. The disorder is characterized by a general retardation in growth, various facial and other somatic abnormalities, and varying degrees of mental retardation (Cicchetti & Beeghly, 1990). During infancy and childhood, those affected are subject to a number of clinical problems and, if they survive beyond the age of 35, their brains invariably show signs of AD. This spectrum of abnormalities is attributable to the presence of an extra copy of chromosome 21, which is due in about 95% of cases to nondisjunction during meiosis (the extra chromosome being derived in about 75% of cases from the mother). The remaining cases are usually due to an unbalanced translocation. There is a strong association between the frequency of the disorder and maternal age. Just how the presence of a third copy of the chromosome leads to this assortment of clinical disorders is unknown, but now that chromosome 21 has been sequenced it seems reasonable to anticipate that substantial progress toward such understanding should soon be forthcoming.

A small (1.5–2.0 Mb) deletion at chromosomal region 7q1.23 is associated with Williams syndrome, a relatively rare developmental disorder affecting perhaps as few as 1 in 20,000 newborns. Children suffering from this disorder tend to have engaging personalities yet display poor social judgment; they are overly friendly not only with known family members and friends but also with complete strangers. Although children with Williams syndrome are clearly retarded (with IQs in the range of 50) and have almost no visuospatial ability, they have surprising language skills and often appear excessively verbal. In addition to their cognitive and affective difficulties (Bellugi, Adolphs, Cassady, & Chiles, 1999), over 75% of the children suffer from cardiac and vascular problems such as aortic and pulmonary artery stenosis. A smaller percentage suffers from various renal abnormalities, strabismus, and orthopedic and gastrointestinal problems. Recent studies of the chromosomal deletion in Williams syndrome have disclosed a cluster of about 15 recognized genes in the region, some of which, like the genes for elastase, a lim kinase (*LIMK1*), syntaxin 1A, and frizzled 9, will probably prove to be associated with the disorder.

There are several chromosomal regions that are commonly referred to as fragile sites. One of these on the X chromosome (at Xq27) is of particular interest in the present context as it is the locus of the major cause of inherited mental retardation in boys, involving 1 in 1,500–2,500 males but less than 1 in 2,000 females. Affected boys show varying degrees of mental retardation and have large ears and large testes. The Fragile X region was cloned and sequenced in 1991 and found to contain an expansion of the trinucleotide repeat CCG (Bell, Hirst, Nakahori, MacKinnon, Roche, Flint, Jacobs, Tommerup, Tranebjaerg, & Froster–Iskenius, 1991; Vincent, Heitz, Petit, Kretz, Oberle, & Mandel, 1991). We shall return later to discuss the function of the relevant Fragile X mental retardation gene (*FMR1*) and protein (FMRP) responsible for Fragile X linked mental retardation, but here we should note that the recognition of the expanded CCG repeats in Fragile X led to the identification of the genes for a number of other trinucleotide repeat disorders including Huntington disease and the spinocerebellar ataxias.

Family, twin, and adoption studies

One of the most informative methods for estimating the contribution of genetics to psychi-

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atric disorders involves the study of families, twins, and adoptees. Rigorous family studies have not only provided convincing evidence in support of the longstanding notion that mental illnesses tend to "run in families" but have also gone well beyond this by providing quantitative estimates of the degree of heritability in many disorders. In an important family study of schizophrenia, Gottesman assessed the clinical status of the family members of a large number of schizophrenic patients (Gottesman, 1991). His major finding was that there is a clear relationship between familial relatedness and the risk of developing the same or a similar illness. Thus, in the case of monozygotic (MZ) twins (who share 100% of their genes), if one twin becomes schizophrenic, the other twin has a 45–50% chance of becoming affected. On the other hand, dizygotic (DZ) twins and other first-order relatives (who share about 50% of their genes) have a substantially reduced risk of developing the illness, averaging around 12%. In second and third degree relatives, such as grandchildren, aunts, uncles, and cousins (who, on average, share approximately 12.5% of their genes), the risk of developing schizophrenia is only about 2%. This is still higher than the risk to the general population (about 1%). The fact that the concordance rate for MZ twins is only 50% or less makes it clear that nongenetic factors contribute to the etiology of the disorder. One measure of this is the observation that the risk to a sibling of an affected individual (about 9%) is greater than the probability that a parent will have the disorder (6%), even though, on average, siblings and parents share the same percentage of genes. The simplest, although by no means the only, explanation is that siblings tend to share the same environments and their environments probably differ significantly from those of their parents.

Twin studies have been especially important in attempts to discern the heritablity of a disorder. Heritability is calculated by taking into account the concordance rates for the disorders between MZ and DZ twins and is usually expressed numerically as twice the difference between the MZ and DZ rates. On the assumption that the environment of all siblings is roughly the same, the difference in rate between MZ and DZ twins is plausibly attributed to genetic factors. Based upon twin concordance estimates, the heritability is thought to be between 60 and 80% for bipolar illness (Tsuang & Faraone, 1990), about 40% for major depression (Sullivan, Neale, & Kendler, 2000), and about 70% for schizophrenia. Although the genetic component or heritability of most mental disorders is substantial, there is no disorder for which MZ twins are 100% concordant, so one must conclude that in every disorder environmental factors are important, whether they operate in utero, infancy, childhood, adolescence, or adulthood.

In 1938 Franz Kallman, followed three to four decades later by Leonard Heston (1970) and Kety and colleagues (Kety, Rosenthal, Wender, Schulsinger, & Jacobsen, 1975), used adoption studies in an attempt to distinguish the contribution of genetic and environmental influences to the pathogenesis of schizophrenia. Such studies complement family and twin studies but have the distinct advantage that the environments in which adoptees are raised are generally different from those of their biological parents. The findings from the Danish and American adoption studies were similar in showing that the biological relatives of a schizophrenic adoptee are significantly more likely to suffer from the same disorder (or, at least, some other mental illness) than are the adoptive family members. Although these studies were criticized on methodological grounds, the essential conclusion is that in schizophrenia the environment in which children grow up is less significant than their genetic susceptibility for the disease.

Genetic linkage analysis

Linkage analysis is a potentially powerful technique for identifying genetic loci associated with mental illness, and in a few instances it has led to the identification of specific polymorphic or mutated genes. The technique is most powerful for detecting single gene disorders (usually referred to as Mendelian disorders) and is less effective where multiple genes are involved, as in com-

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plex disorders like those of greatest interest in psychiatry. The principle underlying linkage analysis is that certain DNA sequences at specific loci are likely to be inherited together because of their proximity to each other on a chromosome. In practice, DNA specimens from both affected and unaffected family members are analyzed, using a variety of known chromosomal markers to identify those that segregate with the illness. A marker that consistently segregates with affected individuals is strongly indicative that the chromosomal region in which it resides contains a gene (or genes) associated with susceptibility for the disorder. The identification of a locus is, of course, distinct from the identification of the actual gene involved. For example, in the case of Huntington disease, it took almost 10 years from the time the locus on chromosome 4 was discovered by linkage analysis to the identification of the Huntington gene. With the sequence data made available by the human genome project and the high throughput sequencers and sequence analysis programs now available, in the future it should take only months rather than years to identify mutated or polymorphic genes once a solid linkage has been identified.

Pedigree studies have also proved useful in identifying linkages involved in the rare familial forms of AD and PD and have led to the discovery of mechanisms that are likely to prove relevant to the more common, sporadic, late onset forms of these disorders. It is interesting to note that many neurodegenerative disorders do not manifest themselves clinically until the latter half of life. Why the cognitive and behavioral phenotypes are not evident earlier is puzzling, particularly because the gene(s) involved appear to be transmitted in an autosomal dominant manner. As to the late onset of neurodegeneration, several ideas have been advanced, including it takes some years for a mutant protein to accumulate to the point that it becomes toxic to the relevant neurons, some trigger (genetic or environmental) occurs later in life and initiates the degenerative process, and a "pathological threshold" is not reached for some time because of the inherent redundancy of function in the system or a large error of margin between the functional threshold of the system and its maximal capacity.

In the common, sporadic cases of PD in which a number of genes have been found to be in allelic association with the disorder (see de Silva, Khan, & Wood, 2000, for review), it is not known whether the responsible genes are less penetrant or only become penetrant following exposure to some environmental factors (see the subsequent discussion of one such environmental factor, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine [MPTP]). The question of *penetrance* is one of concern in neurogenetics as it is in other medical conditions. Penetrance refers to the frequency with which a particular *genotype* manifests itself as an observable *phenotype*. There can be a range of penetrance in different genetic conditions from the fully penetrant to the nondetectable; trying to estimate the degree of penetrance of any particular gene in a multigenic disorder always poses a serious problem.

To date, linkage studies for illnesses such as schizophrenia and bipolar illness, which involve multiple genes and environmental factors, have been disappointing. In part this is because of the difficulties associated with the definition of the phenotypes (as discussed above) and in part to genetic heterogeneity and the challenge of detecting low penetrant, small gene effects (Karayiorgou & Gogos, 1997). Perhaps the best studied disorder from this point of view is schizophrenia, in which the concordance rate for MZ twins is around 50% and the risk to first-degree relatives is only about 10 times greater than that within the general population. This is especially noteworthy as the prevalence of schizophrenia worldwide (1%) is significantly greater than for most Mendelian disorders. The situation is further complicated if some or all of the genes involved interact nonadditively. In such cases many genetic variants may by themselves not significantly increase susceptibility for the disorder and do so only when other interacting variants are present. This does not, of course, rule out the possibility that transmission in some families is due to one or a few dominant loci acting in concert with other small gene effects.

All these and other factors collectively de-

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termine whether an individual who carries the relevant genetic variations manifests the illness and, if so, what form and how severe the illness is likely to be. In the case of bipolar illness, some patients suffer recurring bouts of severe depression alternating with episodes of uncontrollable mania while others suffer only mild depression with infrequent and manageable manic periods. Some individuals respond well to certain medications, whereas the same treatment may be quite ineffective in others. Some patients survive well into old age, but others are driven to early suicide. Until we understand the genetic mechanisms underlying such differences, they are likely to continue to confound both diagnosis and treatment.

The most disappointing aspect of linkage studies in psychiatry is that very few linkages have been reproducible, even when the same patient population has been reexamined. Linkages to schizophrenia and bipolar illness have been identified on almost every chromosome, and the reported loci often span large portions of the identified chromosomes. The most widely used measure for the significance of a proposed linkage is the *lod score* (the logarithm of the odds that the association exceeds chance levels). The strongest linkage to date for schizophrenia (as determined by the lod score) was recently found at the 1q21–q22 chromosomal region, with a lod score of 6.5 (Brzustowicz, Hodgkinson, Chow, Honer, & Bassett, 2000). It is interesting that this region had never before been implicated by linkage analysis in any of the earlier linkage studies of schizophrenia. Conversely, what was originally considered to be evidence for a strong linkage in an Amish pedigree for bipolar illness was found to be no longer significant when other family members were added to the analysis and when new data were incorporated on individuals whose affected status had changed in the meantime (Egeland, Gerhard, Pauls, Sussex, Kidd, Allen, Hostetter, & Housman, 1987; Kelsoe, Ginns, Egeland, Gerhard, Goldstein, Bale, Pauls, Long, Kidd, & Conte, 1989).

Genes

For the past two decades one of the main goals of psychiatric research has been to identify the genes associated with each of the major mental disorders. Once it became clear that there is a genetic component in all these illnesses, most biologically oriented psychiatrists were quick to recognize that it was not sufficient to simply estimate their degree of heritability or discover genetic loci associated with susceptibility to a disorder. They agreed real progress would only be made by identifying and sequencing the actual genes involved and determining the effects of whatever mutations or polymorphisms they display. The considerable successes in this direction in other branches of medicine (including neurology) caused some investigators to be overly optimistic that similar successes would soon be realized in psychiatry. Unfortunately, their optimism has proved to be misplaced. The problems posed by mental illness are considerably greater than had been expected and despite the efforts of several groups in the United States, Europe, and elsewhere, the only genes identified for illnesses that are primarily psychiatric (as opposed to disorders like AD and PD that, although commonly thought of as neurological disorders, have cognitive and affective components) are those for Rett syndrome, autism in families with a history of language disability, and schizophrenia associated with a balanced chromosomal translocation.

The most striking "success story" in the genetics of mental illness is the discovery of the gene for Rett syndrome, an X-linked dominant disorder and the leading cause of mental retardation and autismlike behavior in girls. The hallmark features of the disorder include incessant hand wringing and twisting, clapping, ataxia, and seizures. Unlike most mental illnesses, Rett syndrome has been shown to be due to mutations in a single gene (Amir & Zoghbi, 2000; Shahbazian & Zoghbi, 2001). The gene in question encodes the methyl-CpG-binding protein 2 (MECP2), a transcriptional repressor that normally binds DNA methylated at CpG sites, thereby silencing transcription. It is assumed that the resulting disruption of gene transcription is responsible for the behavioral phenotype.

A second gene associated with a form of autism was recently identified (Wassink,

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Piven, Vieland, Huang, Swiderski, Pietila, Braun, Beck, Folstein, Haines, & Sheffield, 2001). Children from two families with histories of linguistic difficulties were found to have point mutations in the *WNT-2* coding region of chromosome 7q31–33, an area previously linked with autism by a number of groups. In Wassink et al.'s study the individuals suffering from the most severe linguistic impairment also had the highest lod score (3.7). The critical role of the *WNT-2* family of genes in development (including development of the nervous system) is well documented. Supporting evidence has come from a study in which a downstream effector of *WNT-2,* called disheveled (*Dvl1*), was knocked out in mice. The *Dvl1*$^{-/-}$ mice, like autistic children, exhibited impaired social interactions. The knockout animals did not groom each other or huddle with other mice when sleeping, nor did they properly nurture or care for their offspring (Lijam, Paylor, McDonald, Crawley, Deng, Herrup, Stevens, Maccaferri, McBain, Sussman, & Winshaw–Boris, 1997).

In a large Scottish family with a history of schizophrenia and other related illnesses, Porteous and his colleagues recently found a balanced chromosomal translocation involving chromosomes 1 and 11 (Millar, Wilson–Annan, Anderson, Christie, Taylor, Semple, Devon, Clair, Muir, Blackwood, & Porteous, 2000). There are several genes in this region in chromosome 1, and two in particular seem to be critically involved. The first, known as *DISC1* (for disrupted in schizophrenia), appears similar to other genes involved in axonal transport and axon growth. The second, *DISC2,* specifies a noncoding RNA that is antisense to the mRNA of *DISC1* and could serve as a regulator of *DISC1*. The maximum lod score for this locus (6.0) suggests rather that the disruption of one or both of these genes is implicated in the pathogenesis of schizophrenia.

Some of the genes associated with mental retardation have already been alluded to. One that perhaps merits further comment is the trinucleotide CGG repeat expansion found in Fragile X mental retardation. The locus of the disorder was identified on the X chromosome, at Xq 27.3 (Kremer, Pritchard, Lynch, Yu, Holman, Baker, Warren, Schlessinger, Sutherland, & Richards, 1991; Oberle, Rousseau, Heitz, Kretz, Devys, Hanauer, Boue, Bertheas, & Mandel, 1991; Verkerk, Pieretti, Sutcliffe, Fu, Kuhl, Pizzuti, Reiner, Richards, Victoria, Zhang, Eussen, van Ommen, Blonden, Riggins, Chastain, Kunts, Galjaard, Caskey, Oostra, & Warren, 1991) and when this region was cloned the trinucleotide repeat was found to lie in the 5′ untranslated region of exon 1. Normal individuals typically have 6–50 such CGG repeats (with an average of 29); unaffected carriers of the disorder have repeat lengths from about 50 to 200; affected individuals with the full mutation show repeat lengths in excess of 200 up to as many as 1,000. The expanded repeats in Fragile X patients are associated with de novo hypermethylation of an upstream CpG island and transcriptional silencing of the Fragile X (*FMR1*) gene, so that its protein product (FMRP) is not formed. The absence of FMRP is considered to be the direct cause of the Fragile X syndrome phenotype (de Vries, Halley, Oostra, & Niermeijer, 1998). The identification of the trinucleotide repeat in Fragile X led to the discovery of a new mutational mechanism causing disease; and since its report, a whole new group of trinucleotide repeat disorders has been discovered, including spinocerebellar ataxias and Huntington disease. In all of these the triple repeat length is known to increase in successive generations, which accounts for the clinical phenomenon of "anticipation" in which successive generations typically have greater repeat expansions, earlier disease onset, and more severe symptoms.

In contrast to the difficulty of identifying genes in most mental illnesses, the genes involved in disorders that are primarily neurological (but also have cognitive complications) were relatively forthcoming. In the case of early onset AD, three causative genes were discovered (see Selkoe, 2001, for review). First, various mutations were identified in the gene that codes for the β-*amyloid precursor protein* (β-APP; Chartier–Harlin, Crawford, Houlden, Warren, Hughes, Fidani, Goate, Rossor, Roques, Hardy, & Mullan, 1991; Goate, Chartier–Harlin, Mullan, Brown, Craw-

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ford, Fidani, Giuffra, Haynes, Irving, James, Mant, Newton, Rooke, Roques, Talbot, Pericak–Vance, Roses, Williamson, Rossor, Owen, & Hardy, 1991), an important cleavage product of which (β1–40/42) is the major component of the amyloid plaques that are such a distinctive feature of the disorder. Additionally, *Presenilin-1* (*PS1*) was identified by positional cloning after determining a locus on chromosome 14 (Campion, Flaman, Brice, Hannequin, Dubois, Martin, Moreau, Charbonnier, Didierjean, Tardieu, Penet, Puel, Pasquier, Ledoze, Bellis, Calenda, Heilig, Martinez, Mallet, Bellis, Clergetdarpoux, Agid, & Frebourg, 1995; Cruts, Backhovens, Wang, Van Gassen, Theuns, De Jonghe, Wehnert, De Voecht, De Winter, & Cras, 1995), and *presenilin-2* (*PS2*) was recognized on the basis of homology with *PS1* (Sherrington, Froelich, Sorbi, Campion, Chi, Rogaeva, Levesque, Rogaev, Lin, Liang, Ikeda, Mar, Brice, Agid, Percy, Clerget–Darpoux, Piacentini, Marcon, Nacmias, Amaducci, Frebourg, Lannfelt, Rommens, & George–Hyslop, 1996). All early onset genes are transmitted in an autosomal dominant manner. Recent evidence brought the products of these three genes into close association with the discovery that the PS1 protein is probably the so-called γ-secretase involved in the intramembranous cleavage of β-APP. The elucidation of the genetics underlying early onset AD is deservedly recognized as a major advance because it is the first multigenic brain disorder to be understood at this level.

It had been widely believed that the discovery of the genes responsible for the early onset or juvenile forms of neurodegenerative illnesses would lead promptly to an understanding of the much more common late onset and sporadic forms of the disorders. Unfortunately, that has not proved to be true in PD or in amyotrophic lateral sclerosis (ALS), in which the genes associated with the juvenile forms of the disorder, α-synuclein in the case of PD (Kitada, Asakawa, Hattori, Matsumine, Yamamura, Minoshima, Yokochi, Mizuno, & Shimizu, 1998; Polymeropoulos, Lavedan, Leroy, Ide, Dehejia, Dutra, Pike, Root, Rubenstein, Boyer, Stenroos, Chandrasekharappa, Athanassiadou, Papapetropoulos, Johnson, Lazzarini, Duvoisin, Di Iorio, Golbe, & Nussbaum, 1997) and copper-zinc dismutase in ALS (Rosen, Siddique, Patterson, Figlewicz, Sapp, Hentati, Donaldson, Goto, O'Regan, Deng, Rahmani, Krizus, McKenna–Yasek, Cayabyab, Gaston, Berger, Tanzi, Halperin, Herzfeldt, Van der Vergh, Hung, Bird, Deng, Mulder, Smyth, Laing, Soriano, Pericak–Vance, Haines, Rouleau, Gosella, Horvitz, & Brown, 1993), appear to not be involved in the sporadic cases of the illnesses.

In early onset PD (also known as autosomal recessive juvenile Parkinsonism or AR-JP) the clinical symptoms are typically evident before the age of 40 years. AR-JP has been recognized as a distinct genetic and clinical entity for approximately three decades. The first identification of the responsible gene, *Parkin,* was in a Japanese kindred in whom various exons of the gene were found to be missing (Kitada et al., 1998). Since that time, various missense mutations, exon deletions, truncations, point mutations, and protein substitutions were reported in the gene in patients with early onset PD. These mutations appear to be inherited in an autosomal recessive manner.

In late onset PD, mutations in two genes have thus far been identified. These are: α-synuclein and ubiquitin carboxy-terminal hydroxylase (*UCHL1*), both of which are transmitted in an autosomal dominant pattern. The first mutations discovered were missense mutations at two separate sites within the α-synuclein gene (also known as *PARK1*), both of which have been shown to be fully penetrant. The original mutation (Ala53Thr) was found in three Greek families and families of Italian-American heritage (Polymeropoulos et al., 1997). The second mutation (Ala30Pro) was identified in German kindred and firmly established α-synuclein as a gene involved in the pathogenesis of PD (Kruger, Kuhn, Muller, Woitalla, Graeber, Kosel, Przuntek, Epplen, Schols, & Reiss, 1998).

Proteins

As is well known, nearly all genes encode proteins and it is only through changes in the

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affected proteins that genetic mutations exert their "actions." Furthermore, most therapeutic agents used to treat mental illnesses are directed at cellular proteins and commonly act by either enhancing or blocking the actions of certain neurotransmitters or hormones via their specific receptors, downstream effectors, or reuptake mechanisms. Genetic mutations or polymorphisms can result in a variety of aberrant protein products. The affected protein may be misfolded or truncated, or it may contain an expanded repeat or amino acid substitution. In different disorders these changes may result in an abnormal (or even toxic) gain of function or lead to dominant negative effects. Because it is the proteins (and their ability to undergo normal modifications, such as phosphorylation, glycosylation, and, in some cases, lipid modification) that most directly control cellular development and function, in the final analysis all disorders are due to alterations in one or more aspects of protein structure and/or function. Until recently, the analysis of alterations in proteins has been difficult and time consuming. However, the emergence of proteomics (while still lagging behind genomics in terms of technology, current feasibility, and ease of use) should, in the near future, allow for the screening of thousands of proteins for modifications and aberrations that contribute to disease. Next, we highlight the role of aberrant proteins in PD and in Fragile X mental retardation to indicate how the identification of a protein and its function can be critical for understanding pathogenesis.

In PD the various mutations that have been identified in the α-synuclein gene are known to interfere with the proper folding of the protein, thus leading to the formation of filamentous intracellular α-synuclein aggregates or inclusions, which are one of the major components of Lewy bodies (along with proteins that show ubiquitin-like immunoreactivity). Missense mutations in the *UCHL1* gene in an affected German family have also implicated the ubiquitin pathway and proteasomal degradation in PD (Leroy, Boyer, Auburger, Leube, Ulm, Mezey, Harta, Brownstein, Jonnalagada, Chernova, Dehejia, Lavedan, Gasser, Steinbach, Wilkinson, Polymeropoulos, 1998). The function of the ubiquitin pathway is to target and label (ubiquinate) abnormal proteins for destruction. It is thought that the loss of *UCHL1* activity would reduce the degradation of abnormally folded proteins and thereby promote their accumulation within the affected neurons. The Parkin protein, which is involved in juvenile onset PD, is an ubiquitin ligase that functions to physically bind activated ubiquitin with abnormal, damaged, or mutated proteins. These findings have led to the current hypothesis that PD is, at root, a disorder of protein degradation. It is the accumulation of abnormal α-synuclein protein, which is attributable to a hypoactive ubiquitin–proteosomal degradation system within neurons that eventually leads to their degeneration.

In Fragile X, missense mutations in the *FMR1* gene either affect the RNA binding domain (and probably cause a dominant negative effect) or result in a truncated mutation. All these mutations lead to the effective loss of functional FMRP protein. While the precise functions of FMRP are unknown, it has been shown to associate with elongating polyribosomes via large mRNPs, which suggests that it has roles in mRNA transport, translation, and/or stability. An inability to perform these functions and produce proper protein expression in neurons during development could, in time, lead to the observed cognitive and other phenotypic deficits.

Candidate genes and animal models

Because linkage analysis has not yet been particularly successful in identifying genes involved in mental disorders, various alternative approaches have been used. The *candidate gene approach* is one of these. It is based on the assumption (originally derived from the actions of certain therapeutic agents) that particular neurotransmitters or their receptors are likely to be involved in such illnesses. Most of the candidate genes tested thus far are those of the catecholamine neurotransmitter and receptor systems. The serendipitous finding that certain drugs alleviate the symptoms of depression or schizophrenia by actions on the serotonergic, dopaminergic, or noradrenergic

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systems resulted very quickly in a flood of studies looking for changes in these systems. It is disappointing that, none of these studies produced a convincing body of evidence, although the search for mutations and polymorphisms in the relevant transmitters, degradative enzymes, or reuptake mechanism continues unabated.

The creation of transgenic and gene "knockout" animals has provided a powerful means for analyzing the role of particular candidate genes in behavior or disease. In terms of animal models of psychiatric illnesses, one of the best examples to date is that of the *dishevelled 1* (*Dvl1*) knockout mice described above. As noted earlier, the *Dvl1*$^{-/-}$ animals exhibit diminished social interaction, one of the hallmarks of autism. In a subsequent section we consider knockout animal studies that have been important in elucidating the mechanisms of addiction.

Transgenic models of AD have successfully mimicked nearly all of the human neuropathological features and, as can best be determined, some of the more serious behavioral disorders. For example, mice expressing mutated human APP and PS1 display amyloid plaques and associated pathology and show evidence of learning and memory deficits (Borchelt, Ratovitski, van Lare, Lee, Gonzales, Jenkins, Copeland, Price, & Sisodia, 1997; Holcomb, Gordon, McGowan, Yu, Benkovic, Jantzen, Wright, Saad, Mueller, Morgan, Sanders, Zehr, O'Campo, Hardy, Prada, Eckman, Younkin, Hsiao, & Duff, 1998; Hsiao, Chapman, Nilsen, Eckman, Harigaya, Younkin, Yang, & Cole, 1996). It remains a matter of controversy, however, whether the brains of APP/PS1 transgenic animals show the loss of neurons and synapses, a striking feature of human AD. On the other hand, platelet-derived growth factor APP (PDAPP) mice do show synaptic loss and gliosis (Games, Adams, Alessandrini, Barbour, Berthelette, Blackwell, Carr, Clemens, Donaldson, Gillespie, Guido, Hagopian, Johnson–Wood, Khan, Lee, Leibowitz, Lieberburg, Little, Masliah, McConlogue, Montoya–Zavala, Mucke, Paganini, Penniman, Power, Schenk, Seubert, Snyder, Soriano, Tan, Vitale, Wadsworth, Wolozin, & Zhao, 1995), as do the APP23 mice, which have been found to have a degree of neuronal loss (14–25%) that appears to be positively correlated with amyloid plaque levels in the CA1 region of the hippocampus (Calhoun, Wiederhold, Abramowski, Phinney, Probst, Sturchler–Pierrat, Staufenbiel, Sommer, & Jucker, 1998). Similarly, the Tg2576 mice show an 8–10% reduction in hippocampal neurons (Takeuchi, Irizarry, Duff, Saido, Hsiao, Hasegawa, Mann, Hyman, & Iwatsubo, 2000). As yet, no single mouse model of AD has displayed all the features of the human disorder; one feature noticeably missing so far in all the animal models of APP overexpression is the formation of neurofibrillary tangles, which are caused by hyperphosphorylated tau protein. By contrast, both early and late onset AD patients (with APP or PS mutations) invariably show both amyloid plaques and neurofibrillary tangles, which would suggest that the two pathological changes are related in some way.

AD is a particularly good example of how information integrated from the various levels of analysis (from epidemiology to animal models) has advanced our understanding of the disorder. More specifically, epidemiological studies have established that there is a significant association between the presence of the E4 isoform of the apo E protein and the likelihood of late onset AD (Corder, Saunders, Strittmatter, Schmechel, Gaskell, Small, Roses, Haines, & Pericak–Vance, 1993). Furthermore, it was found that regular use of nonsteroidal antiinflammatory agents (ibuprofen, aspirin, etc) appears to reduce the risk of AD (Weggen, Eriksen, Das, Sagi, Wang, Pietrzik, Findlay, Smith, Murphy, Bulter, Kang, Marquez–Sterling, Golde, & Koo, 2001). Additionally, the genotyping of individuals with AD and subsequent allele analysis have indicated that specific polymorphism(s) in the interleukin-1A (IL-1A) and IL-1B genes may increase the risk of developing AD (Du, Dodel, Eastwood, Bales, Gao, Lohmuller, Muller, Kurz, Zimmer, Evans, Hake, Gasser, Oertel, Griffin, Paul, & Farlow, 2000; Nicoll, Mrak, Graham, Stewart, Wilcock, MacGowan, Esiri, Murray, Dewar, Love, Moss, & Griffin, 2000). Individuals homozygous for a specific polymorphism in the

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promoter region of the IL-1A*2 allele have not only an increased risk for AD, but also the age of onset is estimated to be reduced by approximately 9 years (Grimaldi, Casadei, Ferri, Veglia, Licastro, Annoni, Biunno, De-Bellis, Sorbi, Mariani, Canal, Griffin, & Franceschi, 2000). Animal models of the disease have confirmed that treatment with ibuprofen in APP transgenic mice can significantly suppress amyloid plaque formation and the associated inflammation (Lim, Yang, Chu, Chen, Beech, Teter, Tran, Ubeda, Ashe, Frautschy, & Cole, 2000).

Perhaps the most significant development along these lines has been the finding that PDAPP mice treated with a vaccine based on α- and β-amyloid peptide can not only prevent β-amyloid plaque formation and the associated cell loss but also lead to the disappearance of preexisting plaques (Schenk, Barbour, Dunn, Gordon, Grajeda, Guido, Hu, Huang, Johnson–Wood, Khan, Kholodenko, Lee, Liao, Lieberburg, Motter, Mutter, Soriano, Shopp, Vasquez, Vandevert, Walker, Wogulis, Yednock, Games, & Seubert, 1999). Moreover, the β-peptide immunization seems to prevent behavioral impairment and memory deficits in TgCRND8 and APP+PS1 mouse models of AD (Janus, Pearson, McLaurin, Mathews, Jiang, Schmidt, Chishti, Horne, Heslin, French, Mount, Nixon, Mercken, Bergeron, Fraser, George–Hyslop, & Westaway, 2000; Morgan, Diamond, Gottschall, Ugen, Dickey, Hardy, Duff, Jantzen, DiCarlo, Wilcock, Connor, Hatcher, Hope, Gordon, & Arendash, 2000).

Environmental considerations

The contribution of environmental factors to mental illness is undisputed; however, the identification of the factors involved has proved to be very difficult. As briefly mentioned earlier, the discovery that illicit use by addicts of a drug containing MPTP resulted in a disorder clinically indistinguishable from PD (Langston, Ballard, Tetrud, & Irwin, 1983) has provided the most striking evidence for a nongenetic element in PD. The use of MPTP-based animal models of PD has since provided a powerful tool for studying the cause of the focal loss of dopaminergic neurons in the substantia nigra. It has been found in monkeys and mice that the enzyme monoamine oxidase B converts MPTP into MPP^+; MPP^+ is then taken up by dopamine transporters in the caudate-putamen and nucleus accumbens. The uptake of the toxin by the nigrostriatal axons leads, in turn, to the death of neurons in the substantia nigra by impairing the proper functioning of mitochondrial complex I (Javitch, D'Amato, Strittmatter, & Snyder, 1985; Langston, Irwin, Langston, & Forno, 1984; Markey, Johannessen, Chiueh, Burns, & Herkenham, 1984).

A role for the environment on a susceptibility gene is also found in the case of the *ApoE4* gene, whose protein has functions in cholesterol metabolism, transport, and storage. Knowing this, the significant genetic risk of developing late onset AD incurred by having both ApoE4 alleles may potentially be modulated by pharmacological or other intervention measures such as diet and exercise.

Major depression is one psychiatric disorder in which the environment seems to have a critical role in illness onset. The MZ twin concordance rate for major depression is just under 40% (Sullivan et al., 2000), which makes it likely that nonheritable factors play a dominant role in the disorder. Some of the environment factors that have been implicated include hormonal disturbances, obstetric complications, viral or bacterial infections, and a multitude of stressful life events (SLEs).

Many human and animal studies have shown that SLEs seem to precipitate depression. In humans SLEs include personal injury, serious marital problems, divorce or breakup, loss of employment, the death of family members and friends, serious illness, major financial problems, and troubling legal problems. Here, we will only discuss two particular SLEs that have been frequently associated with depression: childhood sexual abuse and spousal physical abuse (Brown & Anderson, 1991; Mullen, Martin, Anderson, Romans, & Herbison, 1993). A recent Maryland-based study showed that individuals who were abused as children show significantly higher mean scores for depression, anxiety, somatization, and interpersonal sensitivity as adults

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(McCauley, Kern, Kolodner, Dill, Schroeder, DeChant, Ryden, Derogatis, & Bass, 1997). Similarly, in a large population of women in London, 64% of those subjected to childhood sexual abuse were depressed at some point during the 3-year study, compared to only 26% in the group that had not experienced such abuse (Bifulco, Brown, & Adler, 1991). In studies in which twin pairs were discordant for exposure to early sexual abuse, the exposed twin had a significantly higher odds ratio (2.43) for the development of major depression than the unexposed twin (Kendler, Bulik, Silberg, Hettema, Myers, & Prescott, 2000).

Meaney and colleagues showed that early life experience can lead to the nongenomic transmission of behavior and stress responses in animal models, which suggests that adverse early life experiences are probably important in determining future stress reactivity and vulnerability to depression (Bremner & Vermetten, 2001; Francis, Diorio, Liu, & Meaney, 1999; Kaufman & Charney, 2001). In terms of hormonal actions, it was demonstrated that hypothalamic–pituitary–adrenal (HPA) responses to stress in adult animals can reflect environmental differences in early life. Adult rats that were handled during the first few weeks of life show reduced ACTH and corticosterone responses to stress compared to nonhandled animals (Meaney, Aitken, van Berkel, Bhatnagar, & Sapolsky, 1988). The handled rats were also found to have an increased concentration of hippocampal glucocorticoid receptors, which is thought to enhance the negative-feedback mechanism associated with stress. The different HPA responses to stress in handled versus nonhandled animals seem to last throughout life.

Variations in maternal care have also been shown to affect the development of the HPA axis and ultimately affect how rats respond to stress later in life. Rat dams that are considered nurturing exhibit high levels of licking and grooming behavior, as well as arched back nursing (LG-ABN). Rat pups raised with high LG-ABN dams showed significantly reduced levels of plasma ACTH and corticosterone in response to restraint stress as adults, compared to pups raised with low LG-ABN dams (Liu, DiOrio, Tanenbaum, Caldji, Francis, Freedman, Sharma, Pearson, Plotsky, & Meaney, 1997). Pups raised by high LG-ABN dams also had increased glucocorticoid mRNA expression, enhanced glucocorticoid feedback sensitivity, and decreased levels of corticotropin releasing hormone mRNA. Cross-fostering studies provide further evidence for a causal relationship between maternal behavior and stress reactivity in the offspring and for the transmission of these differences in maternal behavior from one generation to the next. In addition, an environmental manipulation early in life that alters maternal behavior can affect the pattern of transmission in subsequent generations (Francis et al., 1999).

A particularly striking example of how a simple environmental manipulation can alter the course of a genetic disorder is found in the treatment of phenylketonuria (PKU). PKU is an autosomal recessive disorder due to mutations in the gene encoding the enzyme phenylalanine hydroxylase; as a result, homozygous individuals cannot convert the amino acid phenylalanine to tyrosine (Robson, Chandra, MacGillivray, & Woo, 1982). The accumulation of phenylalanine is toxic and, if affected children are not treated, they suffer devastating and irreversible mental retardation. Since the identification of the gene defect, in developed countries newborns are routinely screened at birth. If infants are found to lack the enzyme, they are then put on a low phenylalanine diet, which by itself prevents the mental retardation. The dramatic success of PKU treatment is rightly considered an important development in human genetics; unfortunately, it is not likely to be replicated in most psychiatric disorders, even in cases where the relevant genes and environmental influences are known. Such treatment will be amenable only in those rare instances, such as in PKU, in which a simple dietary (or other) factor is involved.

Drug Addiction as an Example of the Integration of Approaches at Different Levels

Introduction

Drug addiction imposes a substantial economic and emotional burden to Western society in terms of drug-related deaths, family and

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personal disturbances, and the cost of medical care and law enforcement (Murray & Lopez, 1996). Moreover, it is unfortunate that most current treatments are of limited value, considering the high percentage of addicts who relapse even after long periods of abstinence. In this section we consider drug addiction as one example of a class of disorders that has been analyzed at a number of different levels, but the major focus of the section is current work on the biology of the disorder because it seems likely that the most significant advances are likely to come from molecular and cellular studies using animal models.

Before considering the specific levels of analysis, the following concepts and terms commonly used in addiction research should be clarified. *Drug addiction* is defined as compulsive drug-taking and drug-seeking behavior in the face of severe adverse consequences. Even long after detoxification, addicted individuals report intense cravings for drugs and frequently relapse when exposed to cues that serve as reminders of past drug use or following periods of stress. The most commonly addictive substances include alcohol, psychostimulants (cocaine and amphetamine), opioids, and nicotine. Although each of these drugs has different cellular targets and different modes of action, all addictive drugs are both *rewarding* (in the sense that their actions on the brain are intrinsically positive) and *reinforcing* (i.e., drug-taking behavior tends to be repeated).

With chronic drug use, cellular and molecular adaptations occur within neurons and in the circuits within which they operate. *Homeostatic adaptations* are compensatory in nature and tend to counterbalance the effects of a constantly stimulated target. Such adaptations include altering the presynaptic release of a neurotransmitter or the postsynaptic signaling responses that it elicits; such alterations are probably responsible for the development of *tolerance* (a given dose in time has a diminished effect) and *dependence* (White, 1989). Synaptic plasticity, which is thought to underlie associative learning, may be the cause of cue-dependent relapses, the major clinical problem in the management of addiction (Berke & Hyman, 2000; Hyman & Malenka, 2001).

Epidemiology

The health-related and economic burdens of drug and alcohol addiction are substantial. In Murray and Lopez 1996 report, *Global burden of disease,* alcoholism and drug use are respectively ranked as the 2nd and 10th leading causes of disability in developed nations, and worldwide alcohol use ranks as the 4th major cause of disability. The impact of the disease on individuals is enormous, and its persistence is striking. For instance, in a 33-year follow-up study of 581 male heroin addicts, 284 of the subjects were dead; of the remaining 242 who could be interviewed, 20.7% tested positive for the narcotic and another 9.5% refused to be tested, probably because they were still using the drug (Hser, Hoffman, Grella, & Anglin, 2001). Nicotine is another addictive substance that affects larger numbers of individuals worldwide. The WHO's statistics indicate that nicotine use is on the rise in developing nations and currently causes approximately 4 million deaths each year. In the United States smoking is estimated to contribute to 430,000 deaths per year, and its economic burden is estimated to be about $50 billion (Epping–Jordan, Watkins, Koob, & Markou, 1998). Of particular concern is the limited ability of current treatments to prevent relapse; for instance, the success rate, as measured by continued abstinence in alcoholic individuals, is estimated to be only about 20% (Sibilia, Ceccanti, Deiana, Coriale, & Picozzi, 2001).

The age at which an individual begins to take drugs appears to be critical in determining vulnerability to addiction. Drug use during adolescence (as compared to adulthood) has been shown to be associated with a significantly increased risk of drug addiction. Although the reason for this is unknown, it is possible that the actions of drugs during this unique phase in the development and maturation of the brain may be unusually rewarding and reinforcing (Laviola, Adriani, Terranova, & Gerra, 1999).

Brain pathology

There is no evidence for preexisting or predisposing features in the brain that can be said

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to contribute to drug addiction, but there is some evidence that chronic exposure to drugs can induce morphological changes in neurons. For instance, repeated amphetamine or cocaine treatment has been found to cause increases in the length and density of dendrites and dendritic spines in the nucleus accumbens and prefrontal cortex of rats (Robinson & Kolb, 1997, 1999a). Conversely, chronic morphine treatment is said to induce the pruning of spines and to decrease dendritic length in the nucleus accumbens and neocortex (Robinson & Kolb, 1999b; Sklair–Tavron, Shi, Lane, Harris, Bunney, & Nestler, 1996). How these modifications in synaptic structure (and, ultimately, function) underlie the process whereby repeated drug use leads to addiction remains to be determined.

Excessive alcohol intake during pregnancy is known to cause neurological impairment and cognitive deficits in human infants, a condition known as *fetal alcohol syndrome* (FAS). At autopsy, the brains of affected infants commonly show signs of microencephaly and partial or complete agenesis of the corpus callosum (Clarren, 1986). MRI examination of the brains of children with FAS has similarly revealed discrete changes in the size of the genu and splenium of the corpus callosum (Riley, Mattson, Sowell, Jernigan, Sobel, & Jones, 1995).

Family, twin, and adoption studies

Family, twin, and adoption studies are concordant in their findings that the risk of addiction has a strong genetic basis. Most studies indicate that genetic factors contribute approximately 40–60% of the risk, regardless of the drug of choice. For instance, in twin-family studies of alcoholism in women, the best fitting model suggests that the familial risk for alcoholism is on the order of 51– 59% (Kendler, Neale, Heath, Kessler, & Eaves, 1994). Another US population based registry study of male twins, assessed the lifetime use, heavy use, abuse, and dependence on several substances, including cannabis, sedatives, stimulants, cocaine, opiates, and hallucinogens (Kendler, Karkowski, Neale, & Prescott, 2000). Twin resemblance (a measure of proband concordance and odds ratios among the co-twins of affected individuals) was again found to be largely genetic with an estimated heritability of 60–80%.

A study of children adopted to non-family-related foster parents used data from Stockholm and found that teenage onset and severe alcohol abuse were highly heritable; there was a threefold excess of alcohol abusers among the adopted daughters of alcoholic biological mothers compared to the daughters of nonalcoholic mothers and an excess of alcohol abuse among the daughters of biological fathers who had a history of alcohol abuse (Bohman, Sigvardsson, & Cloninger, 1981; Cloninger, Bohman, & Sigvardsson, 1981). In a follow-up study of this same population done almost two decades later, the findings confirmed that there is a heritable component in juvenile onset and severe alcoholism in male adoptees (Sigvardsson, Bohman, & Cloninger, 1996).

In a US based adoption study, Cadoret, Yates, Troughton, Woodworth, and Stewart (1995) reported a significant relationship between one genetic factor, alcohol abuse or dependency in a biological parent, and an adoptee's drug abuse or dependency. Additionally, they found a strong correlation in both parents and their offspring of what was termed "antisocial personality disorder" and substance abuse and between alcohol abuse or dependency and drug abuse or dependency. Like the findings from family and twin studies, these and other studies of adoptees establish that there is a substantial heritable component to the risk for addiction.

Genetic linkage analysis

Genome-wide searches for genetic linkages associated with addiction have so far been somewhat inconclusive. Certain Asian populations have been found to have variant forms of the genes for alcohol and aldehyde dehydrogenases that may protect such individuals from developing alcoholism. For example, it was found in a Chinese population that homozygosity for the aldehyde dehydrogenase 2 (*ALDH2*2*) allele provides significant protection against alcoholism (Thomasson, Eden-

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berg, Crabb, Mai, Jerome, Li, Wang, Lin, Lu, & Yin, 1991).

In an autosomal genome scan for genetic linkage to alcohol dependence in a Southwestern American Indian tribe, Long, Knowler, Hanson, Robin, Urbanek, Moore, Bennett, and Goldman (1998) identified two suggestive sites on chromosomes 11 and 4, at D11S1984 (lod score 3.1) and D4S3242 (lod 2.8). Of potential interest is the proximity of the chromosome 11 linkage to the DRD4 dopamine receptor and the tyrosine hydroxylase genes and the proximity of the D4S3242 linkage to the β1 GABA receptor gene, all of which have been considered at one time or another to be candidate genes of interest in both drug action and drug addiction.

A six center collaborative study set up in the United States to detect alcohol dependence susceptibility loci identified two suggestive linkages on chromosomes 1 and 7, near D1S1588 (lod 2.93) and D7S1793 (lod 3.49; Reich, Edenberg, Goate, Williams, Rice, Van Eerdewegh, Foroud, Hesselbrock, Schuckit, Bucholz, Porjesz, Li, Conneally, Nurnberger, Tischfield, Crowe, Cloninger, Wu, Shears, Carr, Crose, Willig, & Begleiter, 1998). Evidence was also found from a two-point analysis that there may be a protective locus located on chromosome 4 near the alcohol dehydrogenase gene, as indicated by the earlier Asian population studies.

Animal models of alcohol and drug sensitivity have also proved to be useful in identifying genetic loci that may be implicated. It has long been known that certain strains of rats and mice show greater preferences for alcohol and other drugs than do other strains. For instance, mice of the B6 strain are known to be alcohol "preferrers," whereas D2 mice are extreme alcohol "avoiders." Similarly, different strains of mice have been used to identify loci important in morphine preference (Berrettini, Alexander, Ferraro, & Vogel, 1994; Berrettini, Ferraro, Alexander, Buchberg, & Vogel, 1994). To date, more than 24 large chromosomal regions that appear to contain relevant genes have been implicated in vulnerability to drug addiction by *quantitative trait loci* (QTL; Crabbe, Phillips, Buck, Cunningham, & Belknap, 1999). Each QTL is expected to contain one or more relevant genes but to contribute only a small degree to the total risk of sensitivity to the drug in question. It remains to be determined which of the genes implicated by the above-mentioned linkage and QTL analyses are important in addictive behaviors. For now, one can only conclude that the available data are suggestive but much more work will need to be done for these findings to be wholly convincing.

Genes

With the exception of the *ALDH2*2* gene polymorphism, which in the homozygous state appears to confer protection against alcohol addiction, no specific genes have been identified in humans that unequivocally play a role in the risk of alcohol or drug addiction. However, many candidate genes have been implicated as having a role in addictive behaviors. Among the candidate genes suspected of being involved in addiction are those for many of the molecular targets of addictive drugs and their downstream effectors. Considering that many individuals experiment with drugs at some stage during their lives but only a minority become addicted, it seems likely that certain mutations or polymorphisms in genes that code for neuronal proteins are protective or, alternatively, confer an increased vulnerability to addiction.

The most frequently studied candidate genes have been those that encode monoamine neurotransmitters, neurotransmitter receptors, and transporters; this is because drugs targeted to these systems tend to either alleviate or mimic known psychiatric symptoms. In the following we highlight information on the molecular targets and actions of two classes of drugs, psychostimulants and opiates.

Acutely, psychostimulants increase alertness and produce a sense of well-being. Low drug concentrations usually produce dose-dependent increases in locomotor activity and high concentrations cause stereotypic behaviors. The continued use of psychostimulants usually leads to the development of tolerance or sensitization. Psychostimulants appear to exert these actions by effectively increasing the extracellular concentrations of dopamine,

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norepinephrine, and serotonin in certain regions of the brain, principally in the striatum. Thus, whereas cocaine and amphetamine both target monoamine transporters, their mechanisms of action differ. Cocaine binds to the transporters and in doing so inhibits neurotransmitter reuptake, the result being that the concentration of the relevant monoamine in the synaptic cleft is increased, as is the likelihood of binding to postsynaptic receptors. Amphetamines, on the other hand, also bind with high affinity to monoamine transporters, but they seem to use this to gain access into the presynaptic terminal where they promote the reverse transport of dopamine, norepinephrine, and serotonin, in this way raising the extracellular concentration of the transmitter.

Lesions that interrupt the pathway between the midbrain and the nucleus accumbens (the sites of dopamine synthesis and release, respectively) and the pharmacological blockade of dopamine receptors both indicate that an increase in striatal dopamine is the principal factor mediating the rewarding effects of most drugs. Furthermore, animals in which the dopamine transporter has been knocked out ($DAT^{-/-}$) have been found to be insensitive to the stimulatory effects of cocaine and amphetamine (Giros, Jaber, Jones, Wightman, & Caron, 1996). On the other hand, mice with serotonin ($5\text{-}HT^{-/-}$) and norepinephrine ($NET^{-/-}$) transporter knockouts exhibit normal increases in locomotor behavior when challenged with psychostimulants (Rocha, Scearce–Levi, Lucas, Hiroi, Castanon, Crabbe, Nestler, & Hen, 1998; Xu, Gainetdinov, Westel, Jones, Bohn, Miller, Wang, & Caron, 2000). This does not, however, rule out a role for other catecholamine systems in response to drug administration. For instance, mice lacking the $5\text{-hydroxytryptamine}_{1B}$ receptor ($5\text{-}HT_{1B}^{-/-}$) show a marked decrease in *c-fos* expression (a useful measure of neuronal activity) in various brain structures following cocaine injections (Lucas, Segu, & Hen, 1997). As shown in another study of $5\text{-}HT_{1B}^{-/-}$ mice, there is increased locomotor activity in response to cocaine administration and the animals exhibit enhanced self-administration of the drug (Rocha et al., 1998). Similarly, mice lacking the norepinephrine transporter ($NET^{-/-}$) appear hypersensitive to cocaine and amphetamine as measured by their locomotor activity (Xu et al., 2000).

Various immediate early genes (IEG) and neuropeptides are induced by cocaine or amphetamine treatment. The pattern of expression of many IEGs, and CREB, in response to these drugs, has been well documented (Graybiel, Moratalla, & Robertson, 1990; Konradi, Cole, Heckers, & Hyman, 1994; Moratalla, Robertson, & Graybiel, 1992; Nguyen, Kosofsky, Birnbaum, Cohen, & Hyman, 1992). Some of the changes in the signal transduction pathways and the downstream genes regulated by the IEGs have also been analyzed. For instance, in vitro experiments show that psychostimulants induce the expression of prodynorphin in a phosphorylated CREB- and CRE-dependent manner in the striatum (Cole, Konradi, Douglass, & Hyman, 1995). Moreover, the overexpression of CREB in situ by viral transfection results not only in an increase in the production of dynorphin but also in a decrease in the rewarding actions of cocaine as measured by appropriate behavioral tests (Carlezon, Thome, Olson, Lane–Ladd, Brodkin, Hiroi, Duman, Neve, & Nestler, 1998). In striatal cells NMDA receptors have been found to be critical for *c-fos* expression in response to amphetamine and cocaine administration (Ohno, Yoshida, & Watanabe, 1994; Snyder–Keller, 1991), and it is known also that Ca^{2+} entry following engagement of the NMDA receptors is required for the *c-fos* gene induction (Konradi, Leveque, & Hyman, 1996).

The molecular targets of opiate and opiate-like drugs differ from those of the psychostimulants. They elicit both dopamine-independent and dopamine-dependent rewarding and reinforcing actions. Their dopamine-dependent effects are the result of their binding to opiate receptors on GABAergic interneurons in the ventral tegmental area (VTA) of the midbrain (Johnson & North, 1992). This leads to the secondary effect of an increase in the activity of the dopaminergic projection neurons in the VTA and subsequent dopamine release in the nucleus accumbens. The dopamine-independent effects of the

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drugs, on the other hand, are mediated by opiate receptors in both the locus caeruleus (the major source of noradrenergic fibers in the brain) and the nucleus accumbens itself.

Opiates exert their actions by binding to one of three subtypes of receptor, but most of their rewarding and reinforcing actions appear to be mediated via the so-called *mu* receptor and, to a lesser extent, the *delta* receptors. *Kappa* receptor activation appears to lead to aversive behaviors and is thought to serve as a negative feedback mechanism. When morphine and related drugs bind to the *mu* receptors in the locus caeruleus, they reduce neuronal excitability, primarily by activating a specific K^+ channel and inhibiting a slowly depolarizing Na^+ channel. Chronic opiate use appears to promote compensatory changes in the levels and activities of these channels and the responses they mediate; thus, the cyclic adenosine monophosphate (cAMP) system is upregulated, the Na^+ channel is phosphorylated and promotes increased Na^+ influx, and there is a concurrent decrease in K^+ efflux (Hyman, 1996). Polymorphisms or mutations in the genes encoding the direct targets of these drugs (such as *mu, delta,* or *kappa* opiate receptors or dopamine, serotonin, and norepinephrine receptors and transporters) or their various downstream effectors (G_i, G_s, cAMP, Na^+ channel, K^+ channel, and various transcription factors) are obvious candidates to consider in attempts to understand the genetic basis of addictive behavior.

Although the mechanisms by which different addictive drugs exert their actions are clearly not the same, they all seem to have one action in common: they activate the mesocorticolimbic dopamine system, which appears to be critical for all rewarding and reinforcing behaviors. The ongoing search for genes whose expression in this system changes in response to drugs has included, most productively, the technique known as differential display polymerase chain reaction. Using this method, Hyman and colleagues examined the genes that are induced in the striatum of D1 receptor-supersensitive mice at 3 weeks following the creation of 6-hydroxydopamine lesions that result in virtually complete dopamine depletion. More than 30 genes whose expression changes rapidly in response to a D1 agonist were identified (Berke, Paletzki, Aronson, Hyman, & Gerfen, 1998). Some of these genes (such as *CREM*, *CHOP*, and *MAP* kinase phosphatase-1) are thought to be important in altering homeostatic responses to drug exposure; but other identified genes (including *homer, narp,* and *arc*), as well as certain previously unidentified genes, are probably more important for synaptic remodeling and associative learning, which may lead to addiction. This notion derives from the hypothesis that the compulsions for drugs are due to the usurpation of normal molecular mechanisms responsible for associative types of learning (Hyman & Malenka, 2001).

Proteins

Although we are far from knowing which proteins are involved in drug responses and exactly how they contribute to behavior, a strong conceptual framework is now in place that may lead to a better understanding of drug-related homeostatic and associative learning responses (for a review, see Berke & Hyman, 2000). At present one of the most promising groups of proteins that seem to have a role in modeling behavior and addiction are the members of the Fos family. In animal experiments, acute administration of various addictive drugs has been shown to elicit the rapid induction (within 2 hr) of several transcription factors, including c-Fos, FosB, Fra-1, and Fra-2 in the nucleus accumbens and the dorsal striatum (Hope, Nye, Kelz, Self, Iadarola, Nakabeppu, Duman, & Nestler, 1994; Kelz & Nestler, 2000; Moratalla, Elibol, Vallejo, & Graybiel, 1996). c-Fos is the most rapidly induced protein (typically within 1–4 hr of drug treatment), whereas the Fras show maximal activation 4–8 hr after treatment (Nestler, 2001). In every case, however, the expression of the induced proteins is transient.

Interestingly, after chronic drug treatment, a set of more stable proteins, referred to as "chronic Fras" are expressed; these persist in the brain for relatively long periods of time, on the order of 2 weeks. The chronic Fras are modified isoforms of FosB (ΔFosB); the over-

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expression of ΔFosB in mice increases their sensitivity to the locomotor and rewarding effects of cocaine or morphine (Kelz, Chen, Carlezon, Whisler, Gilden, Beckmann, Steffen, Zhang, Marotti, Self, Tkatch, Baranauskas, Surmeier, Neve, Duman, Picciotto, & Nestler, 1999). Given their long persistence, these proteins represent a potential molecular mechanism through which longer term adaptations and perhaps associative learning in response to chronic drug intake may occur. One target by which ΔFosB may mediate behavioral actions is the AMPA glutamate receptor. In the nucleus accumbens, ΔFosB induces the GluR2 AMPA glutamate receptor subunit, which has the overall effect of reducing neuronal activity. This is consonant with the earlier direct finding of reduced excitability of neurons in the nucleus accumbens following chronic drug exposure (White, Hu, Zhang, & Wolf, 1995).

Several lines of evidence suggest a significant role for the CREB protein in mediating drug responses. For instance, acute and chronic amphetamine and opiate administration have been shown to activate the CREB protein, as measured by increases in pCREB immunoreactivity (Cole et al., 1995). Additionally, indirect evidence suggests that the CREB protein regulates homeostatic responses such as the release of dynorphin from neurons in the nucleus accumbens. Dynorphin binds to a subtype of opiate receptor that seems to be involved in aversive emotions, including anhedonia and dysphoria. It is likely that drug-induced increases in active CREB protein lead not only to increases in dynorphin production but also to its subsequent binding to presynaptic receptors, thereby acting as a negative feedback effector. CREB is also known to play a critical role in synaptic plasticity and the laying down of memories; in these ways it may be an important mechanism through which associative learning can lead to addiction. Other evidence as to the role of CREB protein in drug related behaviors has been derived from transgenic animal models. For example, mice lacking two of the three isoforms of CREB ($CREB_{\alpha\Delta}^{-/-}$, a primary downstream effector of cAMP) show reduced reactions to naloxone-precipitated morphine withdrawal compared to control animals (Maldonado, Blendy, Tzavara, Gass, Roques, Hanoune, & Schutz, 1996). This type of evidence suggests that CREB is involved in opiate-induced transcription of the genes of certain proteins implicated in withdrawal reactions and addiction.

Given that in the brain, genes are generally expressed and proteins only act when the relevant neurons are engaged as functioning components of specific networks, investigators are now attempting to address the problem of drug addiction at the level of the brain circuits within which addicting drugs exert their influences (Kalivas, 2002). This is being studied in several ways, but here we allude only to the current use of functional MRI (fMRI) in the study of addiction. In a fMRI study of the effects of cocaine in the human brain, Breiter and colleagues found that following administration of the drug there were signs of increased activity in several brain areas including the nucleus accumbens, caudate, putamen, basal forebrain, thalamus, insula, and hippocampus (Breiter, Gollub, Weisskoff, Kennedy, Makris, Berke, Goodman, Kantor, Gastfriend, Riorden, Mathew, Rosen, & Hyman, 1997). That the observed changes were related in some fairly direct way to the cocaine administration is borne out by the reproducibility of the findings upon repeated testing. It is interesting that certain brain regions (the ventral tegmentum, pons, basal forebrain, caudate, cingulate, and lateral prefrontal cortex or PFC) showed quick but short duration responses, which may be a reflection of the well-known "rush" that drug users experience. By contrast, other brain regions such as the nucleus accumbens, right parahippocampal gyrus, and some areas of the lateral PFC showed more sustained responses that may be correlated with drug craving.

Conclusion

The major goal of our contribution to this Special Issue was to show that an integrated approach to the study of drug addiction and other psychiatric illnesses promises to be the best way in which to understand the etiology of these disorders and ultimately develop ef-

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fective treatments. As we have tried to demonstrate, each level of analysis, ranging from epidemiology and family and twin studies to linkage analysis and genetics, has strengths and limitations in terms of what it can contribute to the field of developmental psychopathology. It will only be by integrating information from all levels that real progress is likely to be made.

References

Amir, R. E., & Zoghbi, H. Y. (2000). Rett syndrome: Methyl-CpG-binding protein 2 mutations and phenotype–genotype correlations. *American Journal of Medical Genetics, 97,* 147–152.

Baron, M. (2001). Genetics of schizophrenia and the new millennium: Progress and pitfalls. *American Journal of Human Genetics, 68,* 299–312.

Bell, M. V., Hirst, M. C., Nakahori, Y., MacKinnon, R. N., Roche, A., Flint, T. J., Jacobs, P. A., Tommerup, N., Tranebjaerg, L., & Froster–Iskenius, U. (1991). Physical mapping across the Fragile X: Hypermethylation and clinical expression of the Fragile X syndrome. *Cell, 64,* 861–866.

Bellugi, U., Adolphs, R., Cassady, C., & Chiles, M. (1999). Towards the neural basis for hypersociability in a genetic syndrome. *Neuroreport, 10,* 1653–1657.

Berke, J. D., & Hyman, S. E. (2000). Addiction, dopamine, and the molecular mechanisms of memory. *Neuron, 25,* 515–532.

Berke, J. D., Paletzki, R. F., Aronson, G. J., Hyman, S. E., & Gerfen, C. R. (1998). A complex program of striatal gene expression induced by dopaminergic stimulation. *Journal of Neuroscience, 18,* 5301–5310.

Berrettini, W. H., Alexander, R., Ferraro, T. N., & Vogel, W. H. (1994). A study of oral morphine preference in inbred mouse strains. *Psychiatric Genetics, 4,* 81–86.

Berrettini, W. H., Ferraro, T. N., Alexander, R. C., Buchberg, A. M., & Vogel, W. H. (1994). Quantitative trait loci mapping of three loci controlling morphine preference using inbred mouse strains. *Nature Genetics, 7,* 54–58.

Bifulco, A., Brown, G. W., & Adler, Z. (1991). Early sexual abuse and clinical depression in adult life. *British Journal of Psychiatry, 159,* 115–122.

Bohman, M., Sigvardsson, S., & Cloninger, C. R. (1981). Maternal inheritance of alcohol abuse. Cross-fostering analysis of adopted women. *Archives of General Psychiatry, 38,* 965–969.

Borchelt, D. R., Ratovitski, T., van Lare, J., Lee, M. K., Gonzales, V., Jenkins, N. A., Copeland, N. G., Price, D. L., & Sisodia, S. S. (1997). Accelerated amyloid deposition in the brains of transgenic mice coexpressing mutant presenilin 1 and amyloid precursor proteins. *Neuron, 19,* 939–945.

Breiter, H. C., Gollub, R. L., Weisskoff, R. M., Kennedy, D. N., Makris, N., Berke, J. D., Goodman, J. M., Kantor, H. L., Gastfriend, D. R., Riorden, J. P., Mathew, R. T., Rosen, B. R., & Hyman, S. E. (1997). Acute effects of cocaine on human brain activity and emotion. *Neuron, 19,* 591–611.

Bremner, J. D., & Vermetten, E. (2001). Stress and development: Behavioral and biological consequences. *Development and Psychopathology, 13,* 473–489.

Brown, G. R., & Anderson, B. (1991). Psychiatric morbidity in adult inpatients with childhood histories of sexual and physical abuse [See comments]. *American Journal of Psychiatry, 148,* 55–61.

Brzustowicz, L. M., Hodgkinson, K. A., Chow, E. W., Honer, W. G., & Bassett, A. S. (2000). Location of a major susceptibility locus for familial schizophrenia on chromosome 1q21–q22. *Science, 288,* 678–682.

Cadoret, R. J., Yates, W. R., Troughton, E., Woodworth, G., & Stewart, M. A. (1995). Adoption study demonstrating two genetic pathways to drug abuse. *Archives of General Psychiatry, 52,* 42–52.

Calhoun, M. E., Wiederhold, K. H., Abramowski, D., Phinney, A. L., Probst, A., Sturchler–Pierrat, C., Staufenbiel, M., Sommer, B., & Jucker, M. (1998). Neuron loss in APP transgenic mice. *Nature, 395,* 755–756.

Campion, D., Flaman, J. M., Brice, A., Hannequin, D., Dubois, B., Martin, C., Moreau, V., Charbonnier, F., Didierjean, O., Tardieu, S., Penet, C., Puel, M., Pasquier, F., Ledoze, F., Bellis, G., Calenda, A., Heilig, R., Martinez, M., Mallet, J., Bellis, M., Clergetdarpoux, F., Agid, Y., & Frebourg, T. (1995). Mutations of the presenilin I gene in families with early-onset Alzheimer's disease. *Human Molecular Genetics, 4,* 2373–2377.

Carlezon, W. A., Jr., Thome, J., Olson, V. G., Lane–Ladd, S. B., Brodkin, E. S., Hiroi, N., Duman, R. S., Neve, R. L., & Nestler, E. J. (1998). Regulation of cocaine reward by CREB. *Science, 282,* 2272–2275.

Chartier–Harlin, M. C., Crawford, F., Houlden, H., Warren, A., Hughes, D., Fidani, L., Goate, A., Rossor, M., Roques, P., Hardy, J., & Mullan, M. (1991). Early-onset Alzheimer's disease caused by mutations at codon 717 of the beta-amyloid precursor protein gene. *Nature, 353,* 844–846.

Cicchetti, D., & Beeghly, M. (1990). *Down syndrome: A developmental perspective.* New York: Cambridge University Press.

Clarren, S. K. (1986). Neuropathology in fetal alcohol syndrome. In J. R.West (Ed.), *Alcohol and brain development.* New York: Oxford University Press.

Cloninger, C. R., Bohman, M., & Sigvardsson, S. (1981). Inheritance of alcohol abuse: Cross-fostering analysis of adopted men. *Archives of General Psychiatry, 38,* 861–868.

Cole, R. L., Konradi, C., Douglass, J., & Hyman, S. E. (1995). Neuronal adaptation to amphetamine and dopamine: Molecular mechanisms of prodynorphin gene regulation in rat striatum. *Neuron, 14,* 813–823.

Corder, E. H., Saunders, A. M., Strittmatter, W. J., Schmechel, D. E., Gaskell, P. C., Small, G. W., Roses, A. D., Haines, J. L., & Pericak–Vance, M. A. (1993). Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science, 261,* 921–923.

Crabbe, J. C., Phillips, T. J., Buck, K. J., Cunningham, C. L., & Belknap, J. K. (1999). Identifying genes for

https://doi.org/10.1017/S0954579402003036 Published online by Cambridge University Press

alcohol and drug sensitivity: Recent progress and future directions. *Trends in Neuroscience, 22,* 173–179.

Cruts, M., Backhovens, H., Wang, S. Y., Van Gassen, G., Theuns, J., De Jonghe, C. D., Wehnert, A., De Voecht, J., De Winter, G., & Cras, P. (1995). Molecular genetic analysis of familial early-onset Alzheimer's disease linked to chromosome 14q24.3. *Human Molecular Genetics, 4,* 2363–2371.

DeLisi, L. E., Devoto, M., Lofthouse, R., Poulter, M., Smith, A., Shields, G., Bass, N., Chen, G., Vita, A., Morganti, C., Ott, J., & Crow, T. J. (1994). Search for linkage to schizophrenia on the X and Y chromosomes. *American Journal of Medical Genetics, 54,* 113–121.

DeLisi, L. E., Friedrich, U., Wahlstrom, J., Boccio–Smith, A., Forsman, A., Eklund, K., & Crow, T. J. (1994). Schizophrenia and sex chromosome anomalies. *Schizophrenia Bulletin, 20,* 495–505.

de Silva, H. R., Khan, N. L., & Wood, N. W. (2000). The genetics of Parkinson's disease. *Current Opinions in Genetics and Development, 10,* 292–298.

de Vries, B. B., Halley, D. J., Oostra, B. A., & Niermeijer, M. F. (1998). The Fragile X syndrome. *Journal of Medical Genetics, 35,* 579–589.

Du, Y., Dodel, R. C., Eastwood, B. J., Bales, K. R., Gao, F., Lohmuller, F., Muller, U., Kurz, A., Zimmer, R., Evans, R. M., Hake, A., Gasser, T., Oertel, W. H., Griffin, W. S., Paul, S. M., & Farlow, M. R. (2000). Association of an interleukin 1 alpha polymorphism with Alzheimer's disease. *Neurology, 55,* 480–483.

Egeland, J. A., Gerhard, D. S., Pauls, D. L., Sussex, J. N., Kidd, K. K., Allen, C. R., Hostetter, A. M., & Housman, D. E. (1987). Bipolar affective disorders linked to DNA markers on chromosome 11. *Nature, 325,* 783–787.

Epping–Jordan, M. P., Watkins, S. S., Koob, G. F., & Markou, A. (1998). Dramatic decreases in brain reward function during nicotine withdrawal. *Nature, 393,* 76–79.

Francis, D., Diorio, J., Liu, D., & Meaney, M. J. (1999). Nongenomic transmission across generations of maternal behavior and stress responses in the rat. *Science, 286,* 1155–1158.

Games, D., Adams, D., Alessandrini, R., Barbour, R., Berthelette, P., Blackwell, C., Carr, T., Clemens, J., Donaldson, T., Gillespie, F., Guido, T., Hagopian, S., Johnson–Wood, K., Khan, K., Lee, M., Leibowitz, P., Lieberburg, I., Little, S., Masliah, E., McConlogue, L., Montoya–Zavala, M., Mocke, L., Paganini, L., Penniman, E., Power, M., Schenk, D., Seubert, P., Snyder, B., Soriano, F., Tan, H., Vitale, J., Wadsworth, S., Wolozin, B., & Zhao, J. (1995). Alzheimer-type neuropathology in transgenic mice overexpressing V717F beta-amyloid precursor protein. *Nature, 373,* 523–527.

Giros, B., Jaber, M., Jones, S. R., Wightman, R. M., & Caron, M. G. (1996). Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. *Nature, 379,* 606–612.

Goate, A., Chartier–Harlin, M. C., Mullan, M., Brown, J., Crawford, F., Fidani, L., Giuffra, L., Haynes, A., Irving, N., James, L., Mant, R., Newton, P., Rooke, K., Roques, P., Talbot, C., Pericak–Vance, M., Roses, A., Williamson, R., Rossor, M., Owen, M., & Hardy, J. (1991). Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature, 349,* 704–706.

Gottesman, I. I. (1991). *Schizophrenia genesis: The origins of madness.* New York: Freeman.

Graybiel, A. M., Moratalla, R., & Robertson, H. A. (1990). Amphetamine and cocaine induce drug-specific activation of the *c-fos* gene in striosome–matrix compartments and limbic subdivisions of the striatum. *Proceedings of the National Academy of Science, 87,* 6912–6916.

Grimaldi, L. M., Casadei, V. M., Ferri, C., Veglia, F., Licastro, F., Annoni, G., Biunno, I., De Bellis, G., Sorbi, S., Mariani, C., Canal, N., Griffin, W. S., & Franceschi, M. (2000). Association of early-onset Alzheimer's disease with an interleukin-1alpha gene polymorphism. *Annals of Neurology, 47,* 361–365.

Harrison, P. J. (1999). The neuropathology of schizophrenia. A critical review of the data and their interpretation. *Brain, 122* (*Pt. 4*), 593–624.

Heston, L. L. (1970). The genetics of schizophrenic and schizoid disease. *Science, 167,* 249–256.

Holcomb, L., Gordon, M. N., McGowan, E., Yu, X., Benkovic, S., Jantzen, P., Wright, K., Saad, I., Mueller, R., Morgan, D., Sanders, S., Zehr, C., O'Campo, K., Hardy, J., Prada, C. M., Eckman, C., Younkin, S., Hsiao, K., & Duff, K. (1998). Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. *Nature Medicine, 4,* 97–100.

Hope, B. T., Nye, H. E., Kelz, M. B., Self, D. W., Iadarola, M. J., Nakabeppu, Y., Duman, R. S., & Nestler, E. J. (1994). Induction of a long-lasting AP-1 complex composed of altered Fos-like proteins in brain by chronic cocaine and other chronic treatments. *Neuron, 13,* 1235–1244.

Hser, Y. I., Hoffman, V., Grella, C. E., & Anglin, M. D. (2001). A 33-year follow-up of narcotics addicts. *Archives of General Psychiatry, 58,* 503–508.

Hsiao, K., Chapman, P., Nilsen, S., Eckman, C., Harigaya, Y., Younkin, S., Yang, F., & Cole, G. (1996). Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. *Science, 274,* 99–102.

Hyman, S. E. (1996). Shaking out the cause of addiction. *Science, 273,* 611–612.

Hyman, S. E., & Malenka, R. C. (2001). Addiction and the brain: The neurobiology of compulsion and its persistence. *Nature Review Neuroscience, 2,* 695–703.

Janus, C., Pearson, J., McLaurin, J., Mathews, P. M., Jiang, Y., Schmidt, S. D., Chishti, M. A., Horne, P., Heslin, D., French, J., Mount, H. T., Nixon, R. A., Mercken, M., Bergeron, C., Fraser, P. E., George–Hyslop, P., & Westaway, D. (2000). A beta peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease. *Nature, 408,* 979–982.

Javitch, J. A., D'Amato, R. J., Strittmatter, S. M., & Snyder, S. H. (1985). Parkinsonism-inducing neurotoxin, *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine: Uptake of the metabolite *N*-methyl-4-phenylpyridine by dopamine neurons explains selective toxicity. *Proceedings of the National Academy of Science, 82,* 2173–2177.

Johnson, S. W., & North, R. A. (1992). Opioids excite dopamine neurons by hyperpolarization of local interneurons. *Journal of Neuroscience, 12,* 483–488.

Jones, E. G. (1995). Cortical development and neuropathology in schizophrenia. *Ciba Foundation Symposium, 193,* 277–295.

https://doi.org/10.1017/S0954579402003036 Published online by Cambridge University Press

Kalivas, P. W. (2002). Neurocircuitry of addiction. In C. P. O'Brien & M. W. Fischman (Eds.), *Neuropsychopharmacology: The fifth generation of progress* (pp. 1357–1366). Philadelphia, PA: Lippincott Williams & Wilkins.

Kallman, F. J. (1938). *The genetics of schizophrenia.* Locust Valley, NY: J. Augustin.

Karayiorgou, M., & Gogos, J. A. (1997). A turning point in schizophrenia genetics. *Neuron, 19,* 967–979.

Kaufman, J., & Charney, D. S. (2001). The effects of stress on brain structure and function: Implications for understanding the relationship between child maltreatment and depression. *Development and Psychopathology, 13,* 451–471.

Kelsoe, J. R., Ginns, E. I., Egeland, J. A., Gerhard, D. S., Goldstein, A. M., Bale, S. J., Pauls, D. L., Long, R. T., Kidd, K. K., & Conte, G. (1989). Re-evaluation of the linkage relationship between chromosome 11p loci and the gene for bipolar affective disorder in the Old Order Amish. *Nature, 342,* 238–243.

Kelz, M. B., Chen, J., Carlezon, W. A., Jr., Whisler, K., Gilden, L., Beckmann, A. M., Steffen, C., Zhang, Y. J., Marotti, L., Self, D. W., Tkatch, T., Baranauskas, G., Surmeier, D. J., Neve, R. L., Duman, R. S., Picciotto, M. R., & Nestler, E. J. (1999). Expression of the transcription factor deltaFosB in the brain controls sensitivity to cocaine. *Nature, 401,* 272–276.

Kelz, M. B., & Nestler, E. J. (2000). deltaFosB: A molecular switch underlying long-term neural plasticity. *Current Opinion in Neurology, 13,* 715–720.

Kendler, K. S., Bulik, C. M., Silberg, J., Hettema, J. M., Myers, J., & Prescott, C. A. (2000). Childhood sexual abuse and adult psychiatric and substance use disorders in women: An epidemiological and cotwin control analysis. *Archives of General Psychiatry, 57,* 953–959.

Kendler, K. S., Karkowski, L. M., Neale, M. C., & Prescott, C. A. (2000). Illicit psychoactive substance use, heavy use, abuse, and dependence in a US population-based sample of male twins. *Archives of General Psychiatry, 57,* 261–269.

Kendler, K. S., Neale, M. C., Heath, A. C., Kessler, R. C., & Eaves, L. J. (1994). A twin-family study of alcoholism in women. *American Journal of Psychiatry, 151,* 707–715.

Kety, S. S., Rosenthal, D., Wender, P. H., Schulsinger, F., & Jacobsen, B. (1975). Mental illness in the biological and adoptive families of adopted individuals who have become schizophrenic: A preliminary report based on psychiatric interviews. In R. R. Fieve, D. Rosenthal, & H. Brill (Eds.), *Genetic research in psychiatry* (pp. 147–165). Baltimore, MD: Johns Hopkins University Press.

Kitada, T., Asakawa, S., Hattori, N., Matsumine, H., Yamamura, Y., Minoshima, S., Yokochi, M., Mizuno, Y., & Shimizu, N. (1998). Mutations in the Parkin gene cause autosomal recessive juvenile Parkinsonism. *Nature, 392,* 605–608.

Konradi, C., Cole, R. L., Heckers, S., & Hyman, S. E. (1994). Amphetamine regulates gene expression in rat striatum via transcription factor CREB. *Journal of Neuroscience, 14,* 5623–5634.

Konradi, C., Leveque, J. C., & Hyman, S. E. (1996). Amphetamine and dopamine-induced immediate early gene expression in striatal neurons depends on postsynaptic NMDA receptors and calcium. *Journal of Neuroscience, 16,* 4231–4239.

Kremer, E. J., Pritchard, M., Lynch, M., Yu, S., Holman, K., Baker, E., Warren, S. T., Schlessinger, D., Sutherland, G. R., & Richards, R. I. (1991). Mapping of DNA instability at the Fragile X to a trinucleotide repeat sequence p(CCG)n. *Science, 252,* 1711–1714.

Kruger, R., Kuhn, W., Muller, T., Woitalla, D., Graeber, M., Kosel, S., Przuntek, H., Epplen, J. T., Schols, L., & Riess, O. (1998). Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. *Nature Genetics, 18,* 106–108.

Langston, J. W., Ballard, P., Tetrud, J. W., & Irwin, I. (1983). Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science, 219,* 979–980.

Langston, J. W., Irwin, I., Langston, E. B., & Forno, L. S. (1984). 1-Methyl-4-phenylpyridinium ion (MPP+): Identification of a metabolite of MPTP, a toxin selective to the substantia nigra. *Neuroscience Letters, 48,* 87–92.

Laviola, G., Adriani, W., Terranova, M. L., & Gerra, G. (1999). Psychobiological risk factors for vulnerability to psychostimulants in human adolescents and animal models. *Neuroscience and Biobehavioral Reviews, 23,* 993–1010.

Lawrie, S. M., & Abukmeil, S. S. (1998). Brain abnormality in schizophrenia. A systematic and quantitative review of volumetric magnetic resonance imaging studies. *British Journal of Psychiatry, 172,* 110–120.

Leroy, E., Boyer, R., Auburger, G., Leube, B., Ulm, G., Mezey, E., Harta, G., Brownstein, M. J., Jonnalagada, S., Chernova, T., Dehejia, A., Lavedan, C., Gasser, T., Steinbach, P. J., Wilkinson, K. D., & Polymeropoulos, M. H. (1998). The ubiquitin pathway in Parkinson's disease. *Nature, 395,* 451–452.

Lijam, N., Paylor, R., McDonald, M. P., Crawley, J. N., Deng, C. X., Herrup, K., Stevens, K. E., Maccaferri, G., McBain, C. J., Sussman, D. J., & Wynshaw–Boris, A. (1997). Social interaction and sensorimotor gating abnormalities in mice lacking Dvl1. *Cell, 90,* 895–905.

Lim, G. P., Yang, F., Chu, T., Chen, P., Beech, W., Teter, B., Tran, T., Ubeda, O., Ashe, K. H., Frautschy, S. A., & Cole, G. M. (2000). Ibuprofen suppresses plaque pathology and inflammation in a mouse model for Alzheimer's disease. *Journal of Neuroscience, 20,* 5709–5714.

Liu, D., DiOrio, J., Tannenbaum, B., Caldji, C., Francis, D., Freedman, A., Sharma, S., Pearson, D., Plotsky, P. M., & Meaney, M. J. (1997). Maternal care, hippocampal glucocorticoid receptors, and hypothalamic–pituitary–adrenal responses to stress. *Science, 277,* 1659–1662.

Long, J. C., Knowler, W. C., Hanson, R. L., Robin, R. W., Urbanek, M., Moore, E., Bennett, P. H., & Goldman, D. (1998). Evidence for genetic linkage to alcohol dependence on chromosomes 4 and 11 from an autosome-wide scan in an American Indian population. *American Journal of Medical Genetics, 81,* 216–221.

Lucas, J. J., Segu, L., & Hen, R. (1997). 5-Hydroxytryptamine1B receptors modulate the effect of cocaine on *c-fos* expression: Converging evidence using 5-hydroxytryptamine1B knockout mice and the 5-hydroxytryptamine1B/1D antagonist GR127935. *Molecular Pharmacology, 51,* 755–763.

Maldonado, R., Blendy, J. A., Tzavara, E., Gass, P., Roques, B. P., Hanoune, J., & Schutz, G. (1996). Reduction of morphine abstinence in mice with a muta-

https://doi.org/10.1017/S0954579402003036 Published online by Cambridge University Press

tion in the gene encoding CREB. *Science, 273,* 657–659.

Markey, S. P., Johannessen, J. N., Chiueh, C. C., Burns, R. S., & Herkenham, M. A. (1984). Intraneuronal generation of a pyridinium metabolite may cause drug-induced Parkinsonism. *Nature, 311,* 464–467.

McCauley, J., Kern, D. E., Kolodner, K., Dill, L., Schroeder, A. F., DeChant, H. K., Ryden, J., Derogatis, L. R., & Bass, E. B. (1997). Clinical characteristics of women with a history of childhood abuse: Unhealed wounds. *Journal of the American Medical Association, 277,* 1362–1368.

Meaney, M. J., Aitken, D. H., van Berkel, C., Bhatnagar, S., & Sapolsky, R. M. (1988). Effect of neonatal handling on age-related impairments associated with the hippocampus. *Science, 239,* 766–768.

Michaud, C. M., Murray, C. J., & Bloom, B. R. (2001). Burden of disease—Implications for future research. *Journal of the American Medical Association, 285,* 535–539.

Millar, J. K., Wilson–Annan, J. C., Anderson, S., Christie, S., Taylor, M. S., Semple, C. A., Devon, R. S., Clair, D. M., Muir, W. J., Blackwood, D. H., & Porteous, D. J. (2000). Disruption of two novel genes by a translocation co-segregating with schizophrenia. *Human Molecular Genetics, 9,* 1415–1423.

Moratalla, R., Elibol, B., Vallejo, M., & Graybiel, A. M. (1996). Network-level changes in expression of inducible Fos-Jun proteins in the striatum during chronic cocaine treatment and withdrawal. *Neuron, 17,* 147–156.

Moratalla, R., Robertson, H. A., & Graybiel, A. M. (1992). Dynamic regulation of *NGFI-A*(*zif268, egr1*) gene expression in the striatum. *Journal of Neuroscience, 12,* 2609–2622.

Morgan, D., Diamond, D. M., Gottschall, P. E., Ugen, K. E., Dickey, C., Hardy, J., Duff, K., Jantzen, P., DiCarlo, G., Wilcock, D., Connor, K., Hatcher, J., Hope, C., Gordon, M., & Arendash, G. W. (2000). A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. *Nature, 408,* 982–985.

Mullen, P. E., Martin, J. L., Anderson, J. C., Romans, S. E., & Herbison, G. P. (1993). Childhood sexual abuse and mental health in adult life [See comments]. *British Journal of Psychiatry, 163,* 721–732.

Murray, C. J. L., & Lopez, A. D. (1996). *The global burden of disease.* Cambridge, MA: Harvard University Press.

Nelson, M. D., Saykin, A. J., Flashman, L. A., & Riordan, H. J. (1998). Hippocampal volume reduction in schizophrenia as assessed by magnetic resonance imaging: A meta-analytic study. *Archives of General Psychiatry, 55,* 433–440.

Nestler, E. J. (2001). Molecular basis of long-term plasticity underlying addiction. *Nature Review Neuroscience, 2,* 119–128.

Nguyen, T. V., Kosofsky, B. E., Birnbaum, R., Cohen, B. M., & Hyman, S. E. (1992). Differential expression of *c-fos* and *zif268* in rat striatum after haloperidol, clozapine, and amphetamine. *Proceedings of the National Academy of Science, 89,* 4270–4274.

Nicoll, J. A., Mrak, R. E., Graham, D. I., Stewart, J., Wilcock, G., MacGowan, S., Esiri, M. M., Murray, L. S., Dewar, D., Love, S., Moss, T., & Griffin, W. S. (2000). Association of interleukin-1 gene polymorphisms with Alzheimer's disease. *Annals of Neurology, 47,* 365–368.

Oberle, I., Rousseau, F., Heitz, D., Kretz, C., Devys, D., Hanauer, A., Boue, J., Bertheas, M. F., & Mandel, J. L. (1991). Instability of a 550-base pair DNA segment and abnormal methylation in Fragile X syndrome. *Science, 252,* 1097–1102.

Ohno, M., Yoshida, H., & Watanabe, S. (1994). NMDA receptor-mediated expression of Fos protein in the rat striatum following methamphetamine administration: Relation to behavioral sensitization. *Brain Research, 665,* 135–140.

Plum, F. (1972). Prospects for research on schizophrenia: 3. Neurophysiology: Neuropathological findings. *Neuroscience Research Program Bulletin, 10,* 384–388.

Polymeropoulos, M. H., Lavedan, C., Leroy, E., Ide, S. E., Dehejia, A., Dutra, A., Pike, B., Root, H., Rubenstein, J., Boyer, R., Stenroos, E. S., Chandrasekharappa, S., Athanassiadou, A., Papapetropoulos, T., Johnson, W. G., Lazzarini, A. M., Duvoisin, R. C., Di Iorio, G., Golbe, L. I., & Nussbaum, R. L. (1997). Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science, 276,* 2045–2047.

Reich, T., Edenberg, H. J., Goate, A., Williams, J. T., Rice, J. P., Van Eerdewegh, P., Foroud, T., Hesselbrock, V., Schuckit, M. A., Bucholz, K., Porjesz, B., Li, T. K., Conneally, P. M., Nurnberger, J. I., Jr., Tischfield, J. A., Crowe, R. R., Cloninger, C. R., Wu, W., Shears, S., Carr, K., Crose, C., Willig, C., & Begleiter, H. (1998). Genome-wide search for genes affecting the risk for alcohol dependence. *American Journal of Medical Genetics, 81,* 207–215.

Riley, E. P., Mattson, S. N., Sowell, E. R., Jernigan, T. L., Sobel, D. F., & Jones, K. L. (1995). Abnormalities of the corpus callosum in children prenatally exposed to alcohol. *Alcoholism: Clinical and Experimental Research, 19,* 1198–1202.

Robins, E., & Guze, S. B. (1970). Establishment of diagnostic validity in psychiatric illness: Its application to schizophrenia. *American Journal of Psychiatry, 126,* 983–987.

Robins, L. N., & Regier, D. A. (1991). *Psychiatric disorders in America: The Epidemiologic Catchment Area Study.* New York: Free Press.

Robinson, T. E., & Kolb, B. (1997). Persistent structural modifications in nucleus accumbens and prefrontal cortex neurons produced by previous experience with amphetamine. *Journal of Neuroscience, 17,* 8491–8497.

Robinson, T. E., & Kolb, B. (1999a). Alterations in the morphology of dendrites and dendritic spines in the nucleus accumbens and prefrontal cortex following repeated treatment with amphetamine or cocaine. *European Journal of Neuroscience, 11,* 1598–1604.

Robinson, T. E., & Kolb, B. (1999b). Morphine alters the structure of neurons in the nucleus accumbens and neocortex of rats. *Synapse, 33,* 160–162.

Robson, K. J., Chandra, T., MacGillivray, R. T., & Woo, S. L. (1982). Polysome immunoprecipitation of phenylalanine hydroxylase mRNA from rat liver and cloning of its cDNA. *Proceedings of the National Academy of Science, 79,* 4701–4705.

Rocha, B. A., Scearce–Levie, K., Lucas, J. J., Hiroi, N., Castanon, N., Crabbe, J. C., Nestler, E. J., & Hen, R. (1998). Increased vulnerability to cocaine in mice lacking the serotonin-1B receptor. *Nature, 393,* 175–178.

Rosen, D. R., Siddique, T., Patterson, D., Figlewicz,

https://doi.org/10.1017/S0954579402003036 Published online by Cambridge University Press

D. A., Sapp, P., Hentati, A., Donaldson, D., Goto, J., O'Regan, J. P., Deng, H. X., Rahmani, Z., Krizus, A., McKenna–Yasek, D., Cayabyab, A., Gaston, S. M., Berger, R., Tanzi, R., Halperin, J., Herzfeldt, B., Van der Vergh, R., Hung, W.-Y., Bird, T., Deng, G., Mulder, D. W., Smyth, C., Laing, N. G., Soriano, E., Pericak–Vance, M. A., Haines, J., Rouleau, G. A., Gosella, J. S., Horvitz, H. R., & Brown, B. R. (1993). Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature, 362,* 59–62.

Schenk, D., Barbour, R., Dunn, W., Gordon, G., Grajeda, H., Guido, T., Hu, K., Huang, J., Johnson–Wood, K., Khan, K., Kholodenko, D., Lee, M., Liao, Z., Lieberburg, I., Motter, R., Mutter, L., Soriano, F., Shopp, G., Vasquez, N., Vandevert, C., Walker, S., Wogulis, M., Yednock, T., Games, D., & Seubert, P. (1999). Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature, 400,* 173–177.

Selkoe, D. J. (2001). Alzheimer's disease: Genes, proteins, and therapy. *Physiological Reviews, 81,* 741–766.

Shahbazian, M. D., & Zoghbi, H. Y. (2001). Molecular genetics of Rett syndrome and clinical spectrum of MECP2 mutations. *Current Opinion in Neurology, 14,* 171–176.

Sherrington, R., Froelich, S., Sorbi, S., Campion, D., Chi, H., Rogaeva, E. A., Levesque, G., Rogaev, E. I., Lin, C., Liang, Y., Ikeda, M., Mar, L., Brice, A., Agid, Y., Percy, M. E., Clerget–Darpoux, F., Piacentini, S., Marcon, G., Nacmias, B., Amaducci, L., Frebourg, T., Lannfelt, L., Rommens, J. M., & George–Hyslop, P. H. (1996). Alzheimer's disease associated with mutations in presenilin 2 is rare and variably penetrant. *Human Molecular Genetics, 5,* 985–988.

Sibilia, L., Ceccanti, M., Deiana, L., Coriale, G., & Picozzi, R. (2001). Maintenance of abstinence from alcohol. *Lancet, 358,* 1103–1104.

Sigvardsson, S., Bohman, M., & Cloninger, C. R. (1996). Replication of the Stockholm Adoption Study of alcoholism. Confirmatory cross-fostering analysis. *Archives of General Psychiatry, 53,* 681–687.

Sklair–Tavron, L., Shi, W. X., Lane, S. B., Harris, H. W., Bunney, B. S., & Nestler, E. J. (1996). Chronic morphine induces visible changes in the morphology of mesolimbic dopamine neurons. *Proceedings of the National Academy of Science, 93,* 11202–11207.

Snyder–Keller, A. M. (1991). Striatal *c-fos* induction by drugs and stress in neonatally dopamine-depleted rats given nigral transplants: Importance of NMDA activation and relevance to sensitization phenomena. *Experimental Neurology, 113,* 155–165.

Sullivan, P. F., Neale, M. C., & Kendler, K. S. (2000). Genetic epidemiology of major depression: Review and meta-analysis. *American Journal of Psychiatry, 157,* 1552–1562.

Takeuchi, A., Irizarry, M. C., Duff, K., Saido, T. C., Hsiao, A. K., Hasegawa, M., Mann, D. M., Hyman, B. T., & Iwatsubo, T. (2000). Age-related amyloid beta deposition in transgenic mice overexpressing both Alzheimer mutant presenilin 1 and amyloid beta precursor protein Swedish mutant is not associated with global neuronal loss. *American Journal of Pathology, 157,* 331–339.

Thomasson, H. R., Edenberg, H. J., Crabb, D. W., Mai, X. L., Jerome, R. E., Li, T. K., Wang, S. P., Lin, Y. T., Lu, R. B., & Yin, S. J. (1991). Alcohol and aldehyde dehydrogenase genotypes and alcoholism in Chinese men. *American Journal of Human Genetics, 48,* 677–681.

Thompson, P. M., Vidal, C., Giedd, J. N., Gochman, P., Blumenthal, J., Nicolson, R., Toga, A. W., & Rapoport, J. L. (2001). Mapping adolescent brain change reveals dynamic wave of accelerated gray matter loss in very early-onset schizophrenia. *Proceedings of the National Academy of Science, 98,* 11650–11655.

Tsuang, M. T. & Faraone, S. V. (1990). *The genetics of mood disorders.* Baltimore, MD: Johns Hopkins University Press.

Verkerk, A. J., Pieretti, M., Sutcliffe, J. S., Fu, Y. H., Kuhl, D. P., Pizzuti, A., Reiner, O., Richards, S., Victoria, M. F., Zhang, F. P., Eussen, B. E., van Ommen, G. J. B., Blonden, L. A. J., Riggins, G. J., Chastain, J. L., Kunts, C. B., Galjaard, H., Caskey, C. T., Oostra, B. A., & Warren, S. T. (1991). Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in Fragile X syndrome. *Cell, 65,* 905–914.

Vincent, A., Heitz, D., Petit, C., Kretz, C., Oberle, I., & Mandel, J. L. (1991). Abnormal pattern detected in Fragile-X patients by pulsed-field gel electrophoresis. *Nature, 349,* 624–626.

Wassink, T. H., Piven, J., Vieland, V. J., Huang, J., Swiderski, R. E., Pietila, J., Braun, T., Beck, G., Folstein, S. E., Haines, J. L., & Sheffield, V. C. (2001). Evidence supporting WNT2 as an autism susceptibility gene. *American Journal of Medical Genetics, 105,* 406–413.

Weggen, S., Eriksen, J. L., Das, P., Sagi, S. A., Wang, R., Pietrzik, C. U., Findlay, K. A., Smith, T. E., Murphy, M. P., Bulter, T., Kang, D. E., Marquez–Sterling, N., Golde, T. E., & Koo, E. H. (2001). A subset of NSAIDs lower amyloidogenic Abeta42 independently of cyclooxygenase activity. *Nature, 414,* 212–216.

Weissman, M. M., Bland, R. C., Canino, G. J., Faravelli, C., Greenwald, S., Hwu, H. G., Joyce, P. R., Karam, E. G., Lee, C. K., Lellouch, J., Lepine, J. P., Newman, S. C., Rubio–Stipec, M., Wells, J. E., Wickramaratne, P. J., Wittchen, H., & Yeh, E. K. (1996). Cross-national epidemiology of major depression and bipolar disorder. *Journal of the American Medical Association, 276,* 293–299.

White, F. J., Hu, X. T., Zhang, X. F., & Wolf, M. E. (1995). Repeated administration of cocaine or amphetamine alters neuronal responses to glutamate in the mesoaccumbens dopamine system. *Journal of Pharmacology and Experimental Therapeutics, 273,* 445–454.

White, N. M. (1989). Reward or reinforcement: What's the difference? *Neuroscience and Biobehavioral Reviews, 13,* 181–186.

Xu, F., Gainetdinov, R. R., Wetsel, W. C., Jones, S. R., Bohn, L. M., Miller, G. W., Wang, Y. M., & Caron, M. G. (2000). Mice lacking the norepinephrine transporter are supersensitive to psychostimulants. *Nature Neuroscience, 3,* 465–471.

https://doi.org/10.1017/S0954579402003036 Published online by Cambridge University Press