

The Genes and Brains of Mice and Men

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The elucidation of the human genome presents a challenge for psychiatry—determining the impact of thousands of genes on brain functions relevant to mental disorders. For both historical and practical reasons, the mouse has become the mammal of choice for applying molecular genetic approaches to gene function. A working draft of the mouse genome has led to estimates that a mouse version may be identified for 99% of human genes. In accord with their genomic homologies, humans and mice share numerous features of brain organization and behavioral responses to many pharmacological agents. Technologies enabling the precise experimental manipulation of the mouse genome provide unprecedented opportunities for exploring genetic contributions to the regulation of complex behavior and to the pathophysiology and treatment of psychiatric disease. The formidable array of

mouse molecular genetic tools are applied for two general strategies: 1) exploring the function of particular genes by generating lines of mice with precise genetic alterations and 2) searching broadly for those genes that regulate a particular biological trait of interest. Essential to the effective use of these technologies is the implementation of sound strategies for discerning the impact of genetic manipulations on mouse behaviors relevant to psychiatric conditions. These approaches are having a major impact—examples relevant to psychiatric disorders are discussed. However, advances in implementing and interpreting behavioral assays have not kept pace with molecular genetic technologies. To maximize the extent to which the revolution in mammalian genetics may be effectively applied to psychiatric research, new technologies and strategies for mouse behavioral assessment must be developed.

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The elucidation of the human genome will profoundly impact our understanding of human biology. This remarkable achievement enables the identification of the full complement of approximately 30,000 human genes, and it permits new insights into the pathophysiology and treatment of disease. However, genes cannot be systematically manipulated in humans, so we must therefore turn to other organisms to investigate gene function. In recognition of the importance of the mouse as the organism of choice for investigating gene function in the context of mammalian biology, the National Institutes of Health convened a scientific panel that recommended the generation of a “working draft” sequence of the mouse genome by 2003. This deadline was recently met by an international Mouse Genome Sequencing Consortium (1, 2)—an accomplishment that has been heralded as a development of major importance and a boon to investigators working in many areas of biomedical research (3, 4). With the mouse genome sequence in hand, and a formidable array of molecular genetic technologies permitting its manipulation, unprecedented opportunities currently exist to apply these advances to the study of brain function and the physiological underpinnings of psychiatric disorders.

The Ascent of the Mouse

A number of practical and historical considerations have contributed to a rapid escalation in the use of mice for bio-

medical research. As fellow mammals, mice and humans possess similar body plans, organ systems, and mechanisms of physiological regulation. Comparative genomic analyses indicate that the divergence of mammalian lineages giving rise to humans and mice occurred approximately 75 million years ago, a relatively recent event by evolutionary standards (5, 6). The genomes of humans and mice are approximately 2.9 and 2.5 billion nucleotides long, respectively, and both encode approximately 30,000 genes (2). Approximately 99% of mouse genes have human counterparts—conversely, mouse versions (orthologs) can be identified for 99% of human genes. Furthermore, a large proportion of the mouse and human genomes are “syntenic,” i.e., they possess chromosomal regions with the same order of genes. Approximately 96% of mouse genes are found in such syntenic regions. The high level of genomic homology between these species lends support to the view that what distinguishes humans from other mammals relates more to differences in how their genes are regulated and processed than to differences in the identities or numbers of the genes themselves (7, 8).

Historically, the potential benefits of mice have not always been widely appreciated, as reflected by the origin of the word “mouse,” which originated from the Sanskrit “mush,” meaning “to steal” (9). Mice were known to raid grain larders and to spread disease, leading to their status as vermin throughout the Western world. Mice were

viewed more favorably, however, in portions of Asia, where the observation of variants differing in appearance and behavior led to the domestication of unusual mice as pets. Records from 80 B.C. document the existence of mice bred by Japanese mouse enthusiasts who were entertained by their displays of hyperactivity, circling, and head tossing (the behavior of these animals, the likely consequence of inner ear degeneration, led to their later popularization and designation as “waltzing mice”) (9, 10). In the early 19th century, traders transported unusual “fancy” mice from Asia to Europe, where mouse “fanciers” developed many unique varieties by crossing European and Asian subspecies. The popularity of fancy mice in England was illustrated by the establishment of a London “National Mouse Club” that set standards for “show mice” and held contests, awarding prizes for varieties such as white sables, satins, creamy buffs, and ruby-eyed yellows and an overall prize for “best in show” (9, 11, 12).

With the rediscovery of Mendel’s laws in 1900, Harvard biologist William Ernest Castle became interested in the extent to which coat color inheritance in fancy mice resembled the patterns of pea color heritability described by Mendel. Castle obtained animals from the region’s foremost supplier of fancy mice, retired schoolteacher Miss Abbie Lathrop of Granby, Mass., whose mouse farm contained more than 11,000 mice (priced at \$10–\$20 per hundred) (13). Castle’s initial studies of the “experimental evolution” (this preceded the coining of the term “genetics” in 1908) of coat color and his role in training students in this new field earned him recognition as “the father of mammalian genetics.” His students went on to rapidly characterize anomalous phenotypes other than coat color, such as short ears, shaking, hyperglycemia, dwarfism, blindness, and tumor susceptibility (9, 13). Within several years, mice were used by Dr. Ivan Pavlov, who explored genetic influences on behavior by exposing mice to his “dinner bell” in studies of appetitive conditioning (14).

The value of mice for genetic studies has been markedly enhanced by inbreeding strategies for the reduction of genetic heterogeneity. Mice of a particular inbred strain are essentially identical genetically, and they are homozygous (i.e., possessing two identical alleles, one on each homologous chromosome) at every genetic locus. The availability of genetically homogeneous populations of mice is highly beneficial for minimizing the extent to which genetic factors contribute to variability in responses to experimental manipulations. Moreover, each inbred strain possesses a unique, fixed set of alleles resulting in distinct biological properties (also known as “phenotypes”), such as variations in coat color, size, cancer susceptibility, and behavioral traits. Phenotypic differences between inbred strains may be examined in an effort to identify the genetic differences that underlie them. Since the generation of the first inbred mouse strain in 1909, over 450 inbred strains have been developed, contributing to advances throughout biomedical research and to work that has resulted in at least 17

Nobel prizes (11, 15). To this day, most of the strains in common use have ancestors from Abbie Lathrop’s mouse farm (13).

In addition to the reasons just described, practical considerations have also contributed to the popularity of laboratory mice for mammalian genetic studies. Like many small rodent species, they are proficient breeders—their gestation period is 3 weeks, and they are reproductively competent by 6–7 weeks of age. In practice, a breeding program can produce five generations of mice per year, while their small size allows for the economical maintenance of large numbers of animals in group-housing conditions. The development of procedures rendering the mouse genome accessible to experimental manipulation has catalyzed a recent rapid acceleration in the use of mice. Current methods permit the generation of mice bearing mutations of virtually any gene. The elucidation of the mouse genome, coupled with the availability of new molecular technologies enabling examination of genetic influences on behavior, provides unique opportunities to advance psychiatric research. The wide variety of mouse molecular genetic approaches and their application to the study of neural processes relevant to psychiatric illnesses will be discussed.

Relevance of Mouse Behavior to Psychiatric Phenomenology: Our “Inner Mice”

The adult mouse brain is approximately the size of a garbanzo bean—possessing a mass less than 1/2000 that of the human brain. The brains and behavioral patterns of the two species have diverged substantially, in accord with their distinctive ecological niches. The elaboration of the human cerebral cortex and other evolutionary adaptations have contributed to the considerable complexity of human cognitive capacities, affective regulation, social interactions, and societal structures. The relatively modest cortices and communication skills of mice restrict their use as plausible models for psychological processes such as artistic creativity, grief, body image, or dynamic psychotherapy. In light of these obvious species differences, what evidence exists that an understanding of mouse brain function may be pertinent to human behavior and psychiatric disease?

The human cerebral cortex does not function in isolation—it is intimately interconnected with subcortical structures that are well conserved across mammalian species. The brains of vertebrates have a common structural organization, consisting of the cerebral hemispheres, diencephalon, midbrain, cerebellum, pons, and medulla (16). Among mammals, and frequently across other vertebrate classes, the neural structures within these divisions and the circuits that interconnect them have extensive similarities. For example, the substantia nigra appears in reptilian evolution, and this nucleus has a similar organi-

zation among marsupial and placental mammals, including a pars compacta subdivision containing dopaminergic neurons displaying similar patterns of projections to terminal fields (16).

Despite the differing lifestyles of humans and mice, their extensive genetic and neuroanatomical homologies give rise to a wide variety of behavioral processes that are well conserved between species. Exploration of these shared brain functions—our “inner mice”—will shed light on fundamental elements of human behavioral regulation. For example, both humans and mice display complex processes such as hunger, fear, aggression, sleep, circadian rhythms, classical and operant conditioning, and sexual behavior. Functional homologies between species frequently generalize to behavioral responses to drugs—sedative, activating, anorectic, rewarding—and other behavioral properties of drugs observed in humans are frequently found in mice. Such species similarities in behavioral pharmacology are recognized by the pharmaceutical industry, for which rodent behavioral assays are an important component of the psychiatric drug discovery process.

Just as behavioral responses to drugs may generalize across species, so may behavioral responses to genetic perturbations. For example, an X-linked pattern of inheritance was noted in males of a Dutch family for a behavioral disturbance characterized by impulsive aggressiveness and impulsive sexual approaches to females. The syndrome was subsequently attributed to a point mutation in the gene encoding monoamine oxidase A (MAOA) that markedly reduced its function (17). Quite by chance, a similar behavioral syndrome was unintentionally engineered in mice. A line of mice bearing a gene encoding interferon was generated for immunological studies, but investigators observed a phenotype difficult to ascribe to interferon function. When males were group-housed, mutants displayed elevated aggression, resulting in a large number of wounded animals (18). Moreover, the mutant males displayed frequent attempts to grasp and mate with unreceptive females. Further analysis revealed that the interferon transgene had randomly integrated into and disrupted the MAOA gene, leading to a behavioral syndrome mimicking that seen in the Dutch family.

The potential for mouse models to reproduce aspects of complex neuropsychiatric disorders is further illustrated by studies of mutant mice lacking the hypothalamic neuropeptide orexin (19). These animals displayed a dramatic behavioral syndrome, characterized by frequent episodes of inactivity that were manifested by the sudden collapse of the head and buckling of extremities. EEG analysis revealed instances in which the attacks were accompanied by sudden transitions from wakefulness into REM sleep, a phenomenon observed in human narcolepsy and in a strain of narcoleptic Doberman pinschers. Moreover, a mutation of an orexin receptor gene was found to underlie the canine syndrome (20). On the basis of these findings, the orexin system was examined in narcoleptic patients,

and profound orexin deficiencies were observed (21, 22). Thus, studies of orexin-deficient mutant mice revealed a novel role for orexin in the regulation of arousal and an important animal model for examining the pathophysiology and treatment of a neuropsychiatric disorder with complex behavioral manifestations.

These examples illustrate that, in some instances, perturbations of neural genes will produce similar behavioral outcomes in mice and humans. In other cases, however, the consequences of neural mutations will bear little resemblance between species. For example, disparities in behavioral response flexibility attributable to species differences in cortical and other neural specializations could enable humans but not mice to compensate for some mutations of neural genes. Conversely, the neurobehavioral consequences of some mutations may be more readily detected in humans, because of the availability of self-report data and stringent functional requirements imposed on the human nervous system by societal demands. It is notable that species differences in the phenotypic consequences of mutations are not unique to behavior, as evidenced by the marked differences in the lung pathology produced by cystic fibrosis gene mutations across species (23). Despite these discrepancies, mutant mice remain valuable for examining the normal function of this gene and for exploring the genetic interactions and species differences that influence the severity of pulmonary phenotypes. Similarly, examination of factors accounting for species differences in behavioral responses to mutations would provide valuable lines of research.

Modifying the Mouse Genome

The diverse strategies used to modify the mouse genome may be considered to fall within two broad categories. The first includes approaches for introducing known genetic mutations and examining their phenotypic consequences in the resulting animals. Most commonly, transgenic and gene targeting approaches are used to generate lines of mice with enhanced, reduced, or altered gene expression. The second category consists of “phenotype-based” approaches, used for identifying genes that contribute to phenotypic differences observed between inbred strains and to phenotypic abnormalities resulting from the induction of random mutations.

Mice Bearing Mutations of Known Genes

Transgenic technology. Two decades ago, procedures were developed for introducing engineered DNA (“transgenes”) into the mouse genome for the generation of transgenic mice. Thousands of lines of transgenic mice have been generated with this procedure, which has become the most commonly used technique for genetic manipulation in the mouse. Transgenic DNA constructs commonly consist of a gene of interest linked to “promoter” sequences that direct the anatomical distribution and

timing of transgene expression. These constructs are introduced by microinjection into fertilized mouse eggs, which are then surgically transferred to foster mothers. Often, transgenes integrate at a single random chromosomal location in multiple copies, permitting high levels of transgene expression. The resulting transgenic mice may be used as “founders,” which are then bred to transmit the transgene to the next generation. In a small proportion of cases, phenotypes of transgenic mice may result from a transgene inserting into and disrupting the function of a native gene (as was the case for the MAOA mutant described earlier). Controls must therefore be performed to determine the extent to which a phenotype is attributable to the transgene, rather than to its genomic site of integration.

Transgenic techniques may be employed in a wide variety of experimental strategies. Because transgenic mice often possess multiple copies of the transgene, they may be used to examine the consequences of enhancing the function of a particular gene of interest through its “overexpression.” It is also possible to reduce gene function by engineering “dominant negative” mutations that encode proteins designed to interfere with the function of the native gene product. Transgenic procedures may also be used to investigate the roles of particular neuronal cell types by selectively directing expression of genes that alter their function. For example, a transgenic line was developed in which the promoter for the D₁ dopamine receptor was used to drive expression of an activating protein to stimulate cells that express D₁ dopamine receptors. Chronic overstimulation of forebrain neurons expressing D₁ receptors was found to produce an abnormal behavioral phenotype characterized by repetitive grooming and perseverative engagement in other motor patterns that were likened to human compulsive behaviors (24).

Conversely, it is also possible to make cell-type-selective lesions by using DNA constructs in which cell-type-specific promoter sequences are fused to genes encoding toxic proteins. An example of this approach is represented by another line of narcoleptic mice generated to more accurately model human narcolepsy. It has been proposed that the human condition is not typically caused by mutations of orexin or its receptors but is more likely caused by an autoimmune process, resulting in the loss of hypothalamic orexin-containing neurons (25, 26). Thus, the pathophysiology of narcolepsy may involve not only the loss of orexin but also the loss of non-orexin signaling functions of these cells. To examine the consequences of the loss of this population of neurons, a transgenic line of mice was generated in which the human orexin promoter was linked to ataxin-3, a protein that causes cell death. Thus, cells that would normally express orexin were lost in these mutants, resulting in a narcoleptic phenotype that was proposed to more accurately reflect the human condition (27).

Additional strategies are being developed to address a caveat that is frequently pertinent to the interpretation of

transgenic studies: because transgenes are commonly expressed throughout development, the resulting phenotypes could reflect either the adult function of the gene or an indirect consequence of perturbed brain development. To minimize developmental effects, “inducible” gene expression strategies have been developed that permit transgene expression to be activated or suppressed in the adult animal, at times chosen by the experimenter. For example, a line of transgenic mice has been developed with inducible expression of Δ FosB, a transcription factor implicated in behavioral responses to psychostimulants such as cocaine (28). The expression system was designed so that chronic treatment with a tetracycline analog suppressed expression of the transgene. Following cessation of treatment in adult animals, expression levels of Δ FosB rose and were associated with increased behavioral responsiveness to cocaine. Thus, perturbations of development could be excluded as a cause of the cocaine phenotype, strengthening the contention that Δ FosB contributes to the reinforcing properties of cocaine in the normal adult brain. Descriptions of the various strategies used to achieve inducible gene expression may be found in a number of reviews (29–31).

Gene targeting. Another major technical advance, made in the late 1980s, was the development of gene targeting procedures enabling the precise introduction of planned mutations into predetermined sites in the mouse genome. Most frequently, mutations have been designed to generate “knockout” or “null mutant” mice, animals in which the function of an endogenous gene has been completely and selectively eliminated. Gene targeting procedures begin with the introduction of mutation-bearing DNA sequences (targeting constructs) into embryonic stem cells through exposure to an electric field (32). Targeting constructs most commonly consist of a target gene sequence into which a loss-of-function (“null”) mutation has been engineered. They are designed to precisely replace the homologous (matching) native gene sequence within the genome. Embryonic stem cell clones in which this replacement event has occurred are identified and used to generate mice. They are microinjected into the fluid-filled cavity of 3–4-day-old mouse embryos, which are then surgically transferred to surrogate mothers that give birth to “chimeric” mice that are partly derived from the injected embryonic stem cells and partly derived from the host embryos. Chimeras are bred with animals lacking the mutation, and genomic DNA obtained from the progeny is screened for germ line transmission of the mutation. The resulting mice are bred to produce homozygous mutant (bearing two copies of the mutant gene), knockout mice.

Since the initial knockout mice were generated, there has been exponential growth in the number of reported targeted mouse mutants (33). Generation of knockout mice has become part of standard operating procedures for exploring the functions of genes in mammals. In cases

where pharmacological agents that selectively interact with particular gene products are unavailable, examination of knockout mouse phenotypes may be the best method for uncovering their functional significance. Although a number of caveats must be considered in the interpretation of knockout phenotypes (to be discussed), they have frequently provided important insights into gene function and have predicted the actions of drugs (34). To date, null mutations of several thousand genes have been reported, encompassing an estimated 10%–15% of the predicted gene content of the mouse genome and producing a staggering array of phenotypes involving all organ systems (4, 33). Many lines of inbred and knockout mice are maintained by the Jackson Laboratory in Bar Harbor, Me., the world's foremost repository of genetically defined mice. They supply more than 2,500 varieties to the research community and currently list 484 strains with phenotypes relevant to the study of nervous system function.

The potential of knockout mice to shed light on gene functions relevant to behavioral disorders is illustrated by a line of mice lacking the 5-HT_{2C} receptor, a prominent central nervous system serotonin receptor subtype. These animals display a variety of behavioral perturbations, including an eating disorder characterized by chronic elevations of food intake, leading to late-onset ("middle-age") obesity, enhanced susceptibility to type 2 diabetes mellitus, and reduced sensitivity to the anorectic effects of the serotonergic drug dexfenfluramine (35–38). These findings highlighted a role for 5-HT_{2C} receptors in the anorectic effects of serotonin and stimulated efforts to develop 5-HT_{2C} receptor agonists for the treatment of obesity. Further studies revealed that animals lacking this receptor displayed enhanced behavioral and neurochemical responses to cocaine, raising the possibility that 5-HT_{2C} receptor agonists might suppress the intake of psychostimulant drugs, as well as food.

Although most lines of mice generated by gene targeting have been knockouts, alternative strategies employing gene targeting are on the rise. In addition to null mutations, it is possible to introduce more subtle changes, such as point mutations that alter, but do not eliminate, gene function. For example, a single amino acid change was engineered in a gene encoding the α_1 subunit of the γ -aminobutyric acid type A (GABA_A) receptor, rendering GABA_A receptors containing this subunit insensitive to benzodiazepines (39–41). Whereas the resulting animals displayed reduced sensitivity to the sedative and amnestic effects of diazepam, no change in sensitivity to the anxiolytic-like effects of this drug was observed. By contrast, mice bearing a corresponding mutation in the α_2 subunit were insensitive to the anxiolytic-like effects of diazepam. These results indicate a strategy for anxiolytic drug development. Benzodiazepine site ligands active at α_2 -containing GABA_A receptors, while devoid of activity at receptors containing the α_1 subunit, may produce anxiolytic effects

without some of the side effects typically associated with benzodiazepines (41).

Additional advances in gene targeting technologies will allow for cell-type-specific and temporal control of gene expression. In standard knockout mouse lines, the normal gene product is completely absent throughout development from all of the regions in which it is normally expressed. It may therefore be difficult to precisely identify the critical neural circuits through which a mutation alters behavior and the developmental time period in which the mutation produces its effect. To address this problem, "conditional" gene targeting approaches have been devised for the restriction of targeted mutations to subpopulations of cells or for the induction of mutations at predetermined developmental stages. Descriptions of procedures for conditional gene targeting are beyond the scope of this discussion, but recent reviews are available (31, 42).

Phenotype-Based Approaches

In contrast to transgenic and gene targeting approaches, which are often used to explore the function of known genes, phenotype-based approaches work in reverse: phenotypes that exist in particular inbred strains or in animals with induced mutations are subjected to genetic analysis in an effort to identify the genes that contribute to the phenotype. Two approaches in common use are quantitative trait locus and random mutagenesis strategies.

Quantitative trait locus analysis. A quantitative trait locus is a chromosomal region containing a gene (or genes) that contributes a portion of the genetic variation of a quantifiable phenotype. Commonly, mouse quantitative trait locus studies are undertaken to identify "naturally occurring" genetic variations that underlie known phenotypic differences between two inbred strains of mice. For example, one strain of mice may score high and another strain low on a behavioral measure associated with anxiety. Typically, the two strains are interbred, creating a generation of hybrid mice designated F1. The F1 animals are then crossed to produce an F2 generation composed of mice with varying contributions of genes from the two parental strains, due to genetic recombination during gamete formation.

In this example, the F2 mice would then be tested in the anxiety assay that distinguished the two parental strains. A continuous distribution of behavioral scores is usually found, and animals at the extremes of the distribution are selected for further genetic analysis. Correlations are sought between the behavioral scores and the inheritance patterns of genetic markers that are "polymorphic," i.e., that differ between strains. DNA polymorphisms termed "simple sequence length polymorphisms," or "SSLPs," are widely distributed throughout the genome, are readily detected, and may thus serve as markers. Quantitative trait locus analyses are performed by using a variety of statistical techniques to test the probability that variation in the

phenotype is associated with a particular mapped marker. Following identification of quantitative trait loci that contribute significantly to phenotypic variation, a variety of strategies are employed to precisely identify the gene bearing the functional variant. Approaches include analysis of previously unknown genes in the quantitative trait locus region, sequencing of known candidate genes, and determination of differential gene expression. Detailed descriptions of theory and practice are available (43, 44).

Quantitative trait locus analyses allow for the identification of genes influencing phenotypic variation without a priori knowledge of the genes themselves. This is particularly advantageous for the study of complex behaviors, since the genes most relevant to phenotypic variation in neural processes regulating behavior remain unclear. Several limitations to this approach also warrant consideration. For traits that are regulated by a very large number of genes with small effects, very large sample sizes may be required. In addition, quantitative trait locus analysis cannot be used to screen for all genes that are essential to neurobiological pathways regulating behavior. It is restricted to alleles that happen to differ between the two parental strains. Quantitative trait locus analyses have been performed with limited success to identify genes contributing to a large number of neurobehavioral processes, such as anxiety regulation, learning, seizure sensitivity, sensorimotor gating, and responses to drugs of abuse (32).

Random mutagenesis. An alternative phenotype-based genomic screening approach has recently attracted much attention and investment. Efforts are underway to generate large numbers of animals bearing random single-base-pair mutations for screening in a wide variety of phenotypic assays. Mutations are induced chemically, by treating male mice with *N*-ethyl-*N*-nitrosourea to induce single-base-pair mutations in the spermatogonia (45). These mice are then bred, and offspring are screened for phenotypes of interest. Because all mutations in this generation of mice would be in the heterozygous state, phenotypic screening would detect only dominant mutations. To detect recessive mutations (mutations that produce phenotypic abnormalities only in the homozygous state), additional crosses would be required to generate and screen offspring that are homozygous for induced mutations, an expensive task. The doses of *N*-ethyl-*N*-nitrosourea typically employed result in animals with multiple mutations—it has been estimated that 650 lines of the resulting mice are sufficient to obtain animals with null mutations of 15,000 genes (50% coverage of the genome).

The screening of mutagenized mice typically involves assessment in a battery of physiological and behavioral assays. When testing for behavioral phenotypes, it is important to recognize that the induced mutations are random and not restricted to genes regulating the behavioral process of interest (46). For example, genetic perturbations producing illness, motor impairment, cognitive perturbations, blindness, or olfaction deficits could alter be-

havior in an assay intended to assess anxiety. Therefore, tests of peripheral organ system function and a global neurological assessment are usually incorporated in the primary mutagenesis screen. Although many mice are generated by the mutagenesis procedure, practical considerations allow testing of only a small number of mice bearing each unique complement of mutations, limiting statistical power in detecting phenotypic alterations. Therefore, investigators tend to focus on mice with scores near the extremes of the population distribution for the phenotypic assay of interest. Progeny of mice bearing true positive mutations will transmit the altered trait between generations. Once identified, the mutations are localized to chromosomal regions by using gene mapping methods. Ultimately, the actual mutation is identified through demonstration of a sequence difference that tracks with the phenotype.

The potential utility of the random mutagenesis approach has been demonstrated by studies of mice bearing mutations of the *Clock* gene. In a search for genes influencing circadian rhythms, investigators used *N*-ethyl-*N*-nitrosourea mutagenesis and screened animals for genetic influences on wheel-running, a diurnally regulated behavior used to assess circadian rhythmicity. A mutation was found that in the heterozygous state lengthened the circadian period of wheel-running behavior and in the homozygous state led to the loss of circadian rhythmicity altogether (47). The responsible mutation was mapped, and the *Clock* gene was molecularly cloned (48). Further characterization revealed the gene to be expressed in the suprachiasmatic nucleus, a hypothalamic region implicated in circadian rhythm regulation. This work set the stage for studies that are providing novel insights into neural mechanisms that underlie circadian rhythms. Subsequent enthusiasm for chemical mutagenesis approaches has led to the establishment of several international centers devoted to mutagenesis screens (4, 49). It is anticipated that current large-scale efforts will result in thousands of single-gene mutants, many of which will provide novel insights into neural processes that regulate behavior. In addition to chemical methods for inducing mutations, alternative approaches, such as “gene trapping,” are being developed to facilitate identification and characterization of randomly induced mutations (50).

Evaluating Mouse Behavioral Models of Psychiatric Illnesses

The rigorous design and implementation of procedures for analyzing mouse behavior are critical for translating the rapid advances in mammalian genomics into insights relevant to psychiatric disease pathophysiology and treatment. Confusion regarding the interpretation of mouse behavioral tests may be reduced by carefully considering the varying purposes for which particular assays are used. Willner (51) has proposed categorization of behavioral as-

says into three classes: 1) behavioral bioassays, 2) screening tests, and 3) models (simulations) of clinical conditions. Behavioral bioassays utilize behavior as an output measure to assess particular physiological processes. For example, the influence of drugs on the nigrostriatal dopamine system has been assessed by examining their effects on circling behavior in animals that had received unilateral dopamine system lesions. In an analogous fashion, head-twitch responses have been used as a measure of the ability of compounds to act as serotonin receptor agonists. The results of such behavioral bioassays are interpreted with regard to discrete physiological processes rather than to clinical conditions.

Behavioral screening tests are commonly used in the pharmaceutical industry for their “predictive validity”—i.e., the likelihood that the effects of compounds in the assay will predict their efficacy for the treatment of particular psychiatric disorders. A test may be useful for this purpose regardless of whether it appears to accurately reproduce the cause or symptoms of the disorder. For example, the two most frequently used depression-related mouse behavioral tests are the forced swim and the tail suspension “behavioral despair” assays. The forced swim test is conducted by placing animals for several minutes in a water-containing cylinder from which they cannot escape. Initially, mice display high levels of activity in apparent escape attempts, which decrease in frequency as the animals exhibit episodes of immobility during which they appear to float at the surface. This immobile state was initially proposed to reflect “behavioral despair”—the loss of hope of escaping (52). Because immobility in this assay is reduced by a wide variety of antidepressant drugs, the assay is used in the pharmaceutical industry to predict potential antidepressant efficacy of novel compounds. A variant of this assay, the tail suspension test, is more sensitive to serotonergic antidepressants (53). In this test, animals are suspended by the tail for several minutes, and the time spent immobile (without apparent escape attempts) is measured. Mutations of a number of genes implicated in antidepressant action have been associated with abnormal responses in these tests, including those encoding the serotonin 5-HT_{1A} and 5-HT_{1B} receptors, α -adrenergic receptors, monoamine oxidases A and B, and the norepinephrine plasma membrane transporter (53).

Can one conclude that a mouse displaying elevated immobility in these tests is “depressed”? Mice are notoriously noncompliant with questionnaires and interviews, precluding collection of the kinds of self-report data upon which much of psychiatric diagnosis is based. Perturbations of psychological processes must be inferred from behavior, and consideration of the validity of behavioral assays is essential to their interpretation. The “face validity” of the forced swim test, i.e., the degree to which a floating mouse resembles a depressed individual, is limited. It could also be argued that its “construct validity,” i.e., the extent to which the assay reproduces the etiology and

pathophysiology of depression, is also questionable. It is unclear that immobility in this assay reflects a state of “despair,” because immobility may be alternatively viewed as a reasonably adaptive strategy for coping with this experimental situation. In view of these caveats, a conservative interpretation of an elevated immobility result would be warranted. Rather than surmising that the mouse is depressed, it would be more appropriate to conclude that the mouse has an abnormality of a behavior associated with responsiveness to antidepressants. Despite these considerations, the significant predictive validity of the forced swim and tail suspension tests indicates that insights into the mechanisms underlying such a behavioral phenotype may shed light on the function of neural pathways pertinent to the treatment of depression.

Another class of behavioral assays with substantial predictive validity are used to model anxiety states (54). The most frequently employed class of tests assesses exploratory behavior, relying on the innate predisposition of rodents to avoid open and/or brightly lit spaces—presumably an innate response evolved to minimize the risk of predation. For example, when placed in a novel behavioral enclosure, mice exhibit an affinity for the periphery of the behavioral arena rather than the center. The proportion of time spent in the periphery is proposed to correlate with anxiety state. The most commonly used screening test for examining the effects of experimental manipulations on anxiety-like behavior is the elevated “plus” maze. This consists of an elevated platform that is shaped like a plus symbol, with four arms, two of which are walled and two open. The predisposition of mice to prefer the closed to the open arms is proposed to correlate with anxiety state. The effects of pharmacological agents in this assay are predictive of their anxiolytic efficacy in humans. Thus, diazepam increases the proportion of time animals spend exploring the open arms. Conversely, *m*-chlorophenylpiperazine, a nonselective serotonin receptor agonist, reduces exploration of the open arms and produces anxiogenic responses in humans.

To date, behavioral abnormalities consistent with the dysregulation of anxiety have been reported in at least 30 lines of mice (55). For example, marked enhancements of anxiety-related behaviors were observed in three different laboratories that independently generated mice bearing a targeted null mutation of the serotonin 5-HT_{1A} receptor gene (56–58). This phenotype is consistent with the known anxiolytic properties of 5-HT_{1A} receptor partial agonists, such as buspirone. These mutants may be used to examine mechanisms through which serotonin systems regulate anxiety. Behavioral analysis of animals bearing mutations affecting the signaling of corticotropin-releasing factor (CRF) also reveals results consistent with its proposed role in anxiety regulation. Thus, elevated anxiety-like behaviors were observed in mice bearing mutations enhancing CRF expression (59), and reductions of such behaviors were exhibited in mice with genetic perturba-

tions reducing brain CRF signaling (60). Mutations impacting the signaling of acetylcholine, dopamine, GABA, neuropeptide Y, cholecystokinin, nitric oxide, and other neuromodulators have also been found to impact anxiety-related behaviors (55).

It is noteworthy that the assays of rodent depression- and anxiety-related behavior just discussed may be considered to model particular behavioral states rather than the full range of affective, cognitive, and neurovegetative symptoms characteristic of common psychiatric disorders. As discussed in other contributions to this issue, susceptibilities to these illnesses are polygenically determined, and the environmental contributions to their pathophysiology are incompletely understood. Therefore, current mouse models may be most productively used to examine the biological bases of individual features of psychiatric disorders rather than as comprehensive models of complex psychiatric syndromes (54). Exceptions to this are conditions in which clear etiological factors have been identified. In the case of substance use disorders, an important etiological factor, the abused drug, is known. Thus, studies may be performed in which a wide variety of physiological and behavioral responses to the abused substance are examined. In addition, as genetic factors conferring susceptibility to psychiatric diseases are uncovered, it will be possible to perform detailed analyses of the phenotypic consequences of their introduction into the mouse genome.

Priorities for the Development of Neurobehavioral Assessment Strategies in the Mouse

Procedures for the manipulation of the mouse genome are continuing to develop at a rapid pace and are becoming increasingly accessible to investigators. With the development of large-scale mouse mutagenesis programs and the proliferation of inbred, transgenic, knockout, and other genetically modified strains, we have become inundated with valuable mutant mice. The extent to which mouse genetic approaches will provide insights into the neural bases of psychiatric disorders rests critically on the ability to examine the influence of mutations on complex behavior. Unfortunately, technology development for mouse behavioral analysis has lagged behind the pace of innovation in mammalian genetics and genomics. Many of the behavioral assays in common use were originally designed for rats several decades ago and have been recently adapted to mice with little change other than reductions in equipment dimensions. Existing behavioral testing procedures can be time- and labor-intensive, and many factors may complicate their interpretation. These limitations have contributed to a substantial bottleneck in our ability to make maximal use of advances in mouse genome manipulation to study the neural basis of mammalian behavior. The field is currently in its infancy, and its

development would be furthered by progress in a number of areas.

Standardization of Equipment and Experimental Procedures

Currently, many aspects of behavioral testing equipment and procedures are not standardized among laboratories (61). For example, physical features of the elevated plus maze such as dimensions, color, and construction material may differ, contributing to avoidable interlaboratory variability. In addition, procedural differences in the conduct of behavioral assays may vary between laboratories. Often, overlooked variables such as mouse-handling practices, housing conditions, and testing room environments may influence results. Consensus on sets of standard procedures is required, along with enhanced appreciation of the extent to which uncontrolled environmental variables may influence behavioral performance.

Diagnostic Standards

Currently, there are no standards to which investigators can refer to draw conclusions about the behavioral traits of their mutants. As a consequence, some investigators may report a behavioral phenotype based on a single marginal assay, whereas others maintain more stringent criteria. In the absence of clear diagnostic standards, a conservative approach would be to require a consistent pattern of abnormal behavioral responses across several assays pertinent to a given behavioral domain before conclusions are drawn.

Need to Assess Multiple Behavioral Domains

Principles of clinical evaluation can be useful in the analysis of mutant mouse phenotypes. For example, clinicians do not limit their inquiries to the chief complaint, and they perform a review of systems to minimize the risk of overlooking important information. However, investigators interested in a particular behavioral trait sometimes perform a very restricted analysis, limited to the behavioral domain of interest. This could be problematic because an undetected deficit in another behavioral domain could influence the interpretation of results. For example, an animal with normal trait anxiety could perform abnormally on the elevated plus maze because of an undetected cognitive deficit. Conversely, a mouse with a motor impairment or a severe stress response to a learning task may perform abnormally for reasons other than cognitive impairment. Thus, the exploration of multiple behavioral domains will maximize the extent to which each individual assay may be correctly interpreted.

Limitations of Behavioral Batteries

To maximize the information that can be obtained from limited numbers of mutant5, cohorts of mice are often examined in a battery of behavioral tests requiring repeated removal from their home cages. Implementation of behavioral batteries may be associated with drawbacks that

are difficult to avoid, such as 1) they are time-consuming and labor-intensive, 2) the order of test administration can skew the resulting data, and 3) repeated removal of mice from the home cage produces stress that may confound interpretation of behavioral data. These problems may be addressed by use of experimental designs that control for test order and by the development of alternative behavioral analysis strategies permitting simultaneous assessment of multiple behaviors, as will be described.

Strain Information

The large number of available inbred strains represents a resource that has yet to be fully utilized. Although inbred strains are known to display a wide variety of behavioral phenotypes, these have not been systematically characterized. To address this issue, a large-scale international "Mouse Phenome Project" has been recently initiated by the Jackson Laboratory to establish a database containing detailed phenotypic information (behavioral and nonbehavioral) from a wide variety of inbred strains (62). Such information may be used for the purpose of selecting strains with characteristics most suitable for investigating particular mutant phenotypes or for identifying strain differences in traits of interest for quantitative trait locus studies.

Need for Assays of Additional Behaviors

The development of satisfactory animal models that simultaneously mimic multiple features of complex psychiatric disorders of uncertain etiology may be extremely difficult. However, it may be feasible to develop new assays relevant to particular features of psychiatric illnesses that are not commonly modeled in mice, such as compulsions, panic attacks, binge eating, impulsivity, distractibility, and anhedonia. In some cases, useful assays that have been previously established in rats could be adapted to mice. In other cases, novel approaches will be required.

Gene-Environment Interactions

Susceptibility to psychiatric illnesses depends not only on genetic endowment but also on experience. Although mouse genetic studies most commonly focus on the influences of genes, they may also be used to explore the interactions between genes and environment on the establishment of behavioral traits. For example, rodent behavior is susceptible to social influences, as demonstrated by studies revealing that a mother's treatment of her pups can produce lifelong influences on stress reactivity in her offspring (63). Mouse molecular genetic approaches may be applied to determine the influence of genes both on maternal behavior and on the sensitivity of pups to experimental perturbations of the maternal care they receive. Genetic influences on the behavioral consequences of a wide range of additional environmental factors, including chronic stress, social defeat, diet, and environmental enrichment, also warrant further exploration in the mouse.

Behavioral Assays Applied to Both Humans and Mice

A challenge in determining the relevance of animal studies to psychiatric conditions results from fundamental differences in the nature of the data used for assessment of psychological processes in humans and mice. While psychiatric assessment relies heavily on self-report data, assessment of psychological processes in mice requires inferences derived from the analysis of behavior. Although the prospects of obtaining useful self-report data from mice remain discouraging, there is increasing interest in the development of behavioral assays that may be applied to both mice and humans. One example is the prepulse inhibition assay, which examines the ability of a sensory stimulus to suppress the startle response to a subsequent stimulus. This index of sensorimotor gating is perturbed in schizophrenia, and the effects of drugs on prepulse inhibition are similar in mice and human subjects (64). Many possibilities exist for the development of new cross-species assays that may be applied to additional domains of behavior.

Need for Technological Innovations for Behavioral Assessment

New technologies that have revolutionized genomics and other scientific fields may also be used to develop novel approaches for behavioral assessment—the application of advances in information technology may be particularly useful. Toward this end, my colleagues and I, as well as others, are combining automated behavioral data collection systems with sophisticated computational tools for "behavioral informatics" approaches to phenotype analysis. The spontaneous behavior patterns exhibited by mice in their home cages provide a rich source of information reflecting the functional output of the brain. Behaviors such as exploration, feeding, drinking, sleeping, grooming, and diurnal rhythms reflect the functions of numerous neuronal pathways, each influenced by large numbers of genes. Rather than removing animals from their home cages and isolating various behavioral domains in individual tests, this approach will entail the introduction of experimental manipulations into the home cage. Their impact may thus be examined in the context of the integrated expression of multiple behavioral domains ("mouse lifestyles"), reflecting the outputs of multiple neuronal pathways. We have been developing such technology with the goal of systematically establishing a database recording the impact of genes, drugs, environmental exposures, and brain lesions on spontaneous behavioral patterns. Such a resource will provide a sensitive tool for assessment of the neurobehavioral consequences of mutations and other experimental manipulations. We anticipate many such new technology initiatives will be developed in academic and industrial settings. Such efforts, along with progress in meeting the multiple challenges already outlined, will permit a detailed assessment of brain

function in the mouse and enhance the extent to which the revolution in mouse molecular genetics will benefit psychiatric research.

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