Forward and Reverse Genetic Approaches to Behavior in the Mouse

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Modern molecular genetic and genomic approaches are revolutionizing the study of behavior in the mouse. "Reverse genetics" (from gene to phenotype) with targeted gene transfer provides a powerful tool to dissect behavior and has been used successfully to study the effects of null mutations in genes implicated in the regulation of long-term potentiation and spatial learning in mice. In addition, "forward genetics" (from phenotype to gene) with high-efficiency mutagenesis in the mouse can uncover unknown genes and has been used to isolate a behavioral mutant of the circadian system. With the recent availability of high-density genetic maps and physical mapping resources, positional cloning of virtually any mutation is now feasible in the mouse. Together, these approaches permit a molecular analysis of both known and previously unknown genes regulating behavior.

Complex as it is, much of the vast network of cellular functions has been successfully dissected, on a microscopic scale, by the use of mutants in which one element is altered at a time. A similar approach may be fruitful in tackling the complex structures and events underlying behavior, using behavioral mutations to indicate modifications of the nervous system.

—SEYMOUR BENZER (*1*, p. 1112)

Although complex behaviors are generally assumed to be under polygenic control, a handful of behaviors, especially among invertebrates, are profoundly regulated by single genes. In research pioneered by Benzer and colleagues in *Drosophila* (*2*, *3*), examples of such genes include those influencing learning and memory (*4*), courtship behavior (*5*), and circadian rhythms (*6*, *7*). At the same time, mouse genetics has had a longstanding tradition in behavioral analysis (*8*). Unlike the mutagenesis and screening approach favored in *Drosophila*, however, most mouse behavioral genetics has depended on natural variants and spontaneous mutants. Because genetic screens for specific behaviors have been rarely undertaken in the mouse, most neurological or behavioral mutants have obvious phenotypes (such as neuromuscular defects) or involve pleiotropic effects of coat color mutations (*9*). The cloning of such mutations was until recently a serendipitous endeavor, at best.

Recently, however, both forward and reverse genetic approaches have become feasible in the mouse. The revolution in transgenic (*10–12*) and gene targeting methods (*13*, *14*) has opened the way for reverse genetic approaches to the study of behavior. Furthermore, forward genetic approaches have been developed in the mouse (*15–18*), and it is now feasible to isolate mutations of a desired class by chemical mutagenesis and screening procedures (*19–21*). The mapping and molecular identification of such induced mutations is now tractable with the development of high-density genetic linkage maps (*22–25*) and with the availability of substantial physical mapping and cloning resources in the mouse (Boxes 1 and 2) (*25*, *26*). In many ways, mouse genetics now is at the threshold of discovery of behavioral mechanisms just as *Drosophila* genetics was in the 1970s. Both forward and reverse genetic approaches can be successfully applied to the mouse to analyze behavior.

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Gene Targeting Approaches to Behavior

Reverse genetic approaches, in which mutations are produced by replacement of a gene of choice (*13*, *14*, *27*, *28*), have been used extensively to study development and pattern formation in the mouse (*29*); however, their use in the analysis of behavior has only recently begun. Of the 250 mutant strains that have been produced by gene targeting (*28*), a small but increasing number of mutations have been made in genes whose functions have been implicated in various aspects of neuronal signaling (Table 1). The use of "gene knockouts" to study behavior was pioneered by the laboratories of Tonegawa and colleagues (*30*, *31*) and Kandel and colleagues (*32*) to analyze the role of serine-threonine and tyrosine kinases in the regulation of synaptic plasticity and forms of learning and memory that involve the hippocampus. On the basis of extensive behavioral and physiological work, the hippocampus has been implicated in "declarative" rather than "procedural" types of memory tasks in mammals (*33*). In rodents, "spatial" learning and memory tasks critically depend on the hippocampus, and a role for *N*-methyl-D-aspartate (NMDA) receptors has been strongly implicated in mediating these behaviors (*34*).

In addition, the hippocampus has been shown to express a number of forms of use-dependent synaptic plasticity (*35–39*). These include: (i) short-term potentiation (STP), which is an activity-dependent increase in synaptic efficiency that lasts less than 1 hour; (ii) long-term potentiation (LTP), which is a long-lasting, activity-dependent increase in synaptic efficiency that shows cooperativity, associativity, and input-specificity; (iii) post-tetanic potentiation (PTP) and paired-pulse facilitation (PPF), which are increases in synaptic efficiency that have very short time courses and are general features of excitatory transmission; and (iv) long-term depression (LTD), which is an activity-dependent decrease in synaptic efficiency (*37*, *39*). The LTP in the CA1 subdivision of the hippocampus is a primary model for activity-dependent synaptic plasticity in the mammalian central nervous system (*37*). The induction of LTP in CA1 is postsynaptic and requires NMDA receptor activation and an elevation of intracellular $[Ca^{2+}]$ (*37*). A number of protein kinases have also been implicated in the induction of LTP, including protein kinase C (PKC), Ca^{2+}-calmodulin–dependent protein kinase II (CaMKII), and tyrosine kinases (*37*). The maintenance of LTP appears to involve a presynaptic increase in transmitter release, which requires a "retrograde messenger"; nitric oxide (NO) appears to be the best candidate for this (*40*). In addition, LTP in CA1 can be subdivided into an early phase of LTP, which is triggered by one train of high-frequency stimulation and that lasts for 2 to 3 hours and is blocked by kinase inhibitors but not by protein synthesis inhibitors, versus a later phase of LTP, which is triggered by multiple trains of high-frequency stimulation and that lasts for many hours and requires protein synthesis (*37*). Evidence suggests that adenosine 3′,5′-monophosphate (cAMP) and activation of cAMP-dependent protein kinase (PKA) may be components that lead to the later phase of LTP (*41*).

The participation in LTP of three different protein kinase genes, the α isoform of

CaMKII (α-CaMKII), the nonreceptor-type tyrosine kinase *fyn*, and the γ isoform of protein kinase C (PKC-γ), has been analyzed by reverse genetics. Gene knockouts of α-CaMKII and Fyn both strongly impair the production of LTP but do not completely eliminate it, which suggests that these kinases are regulatory rather than essential in LTP (*30, 32*). Mutations of other nonreceptor-type tyrosine kinases (*src, yes, abl*) do not interfere with either the induction or maintenance of LTP (*32*). Interestingly, mutations of α-CaMKII and Fyn also impair hippocampal-dependent spatial learning behaviors without affecting tasks that require a nonspatial association (*31, 32*). Thus, in these gene knockouts an additional correlation between hippocampal LTP and spatial learning behavior exists. In addition, PKC has long been implicated in the induction of LTP (*42*). The expression of the PKC-γ isoform is brain-specific and is enriched in the hippocampus (*42*). A null mutation of the gene encoding PKC-γ also blunts LTP but does not affect another form of synaptic plasticity, LTD (*43*). In addition, prior treatment of hippocampal slices with low-frequency stimulation that produces LTD enables LTP to be subsequently produced in PKC-γ knockouts (this type of LTP is called "primed LTP"). This priming effect clearly

Box 1. Glossary of the mouse genome

Inbred strain. A set of animals that is produced by at least 20 consecutive generations of sister × brother or parent × offspring mating and that can be traced to a single ancestral pair in the 20th or subsequent generation. Animals of an inbred strain are nearly fully homozygous, which thus provides a defined and consistent genotype for analysis (*128, 129*).

Congenic strain. A strain that differs from another in the region of one genetic locus and that is produced by at least 10 successive backcrosses or intercrosses to the control strain. These mice can be used to evaluate a mutation or allelic variant against a particular inbred strain background (*130*).

RI (recombinant inbred) strains. A set of strains originating from the second generation of intercrossing of two inbred strains, then inbreeding different lines at least 20 generations. Each RI strain has a different, but reproducible, recombined sampling of the genomes of the inbred strains of origin (*131, 132*).

Transgenic mice. These can be made by microinjection of DNA into the pronucleus of fertilized eggs, with the DNA integrating at random. They are useful for studying gene expression, overexpression, and rescue of the mutant phenotype (*133*).

Targeted gene transfer. With the method of homologous recombination, or gene replacement, targeted gene transfer can create knockouts and other specific mutations (*13, 14*).

Genetic map. A map based on the segregation of genes or DNA markers in a cross. Units of genetic distance are based on recombination rates—that is, centimorgans (cM).

Linkage of two genes is based on a greater association than expected from an independent assortment of chromosomes. It indicates that the genes are on the same chromosome and provides for an estimation of genetic or relative distance. The degree of linkage can be statistically described by the logarithm of the ratio (probabilities of linkage/probability of no linkage), called the logarithm of the odds (lod) score.

Haplotype analysis is a method of inferring the relative position of genes or DNA markers assuming a minimum number of crossovers have occurred along the chromosome.

RFLPs (restriction fragment length polymorphisms) are DNA fragments of different length generated by cleaving with a specific endonuclease; they can be used for genetic mapping, particularly in interspecific crosses (*134*).

SSLPs (simple sequence length polymorphisms) are variations in the number of repeats of a simple sequence such as [CA]; they are also known as microsatellites. Such polymorphisms have been exploited as DNA markers for intraspecific crosses: with PCR primers designed for the unique sequence flanking the repeat, size differences can be detected in PCR products (*22, 23, 135*).

SSCPs (single-strand conformation polymorphisms) are sequences that differ by as little as a single base and that can be detected by the migration of short, single-stranded fragments in nondenaturing gels. SSCPs of PCR products can be used as markers for linkage analysis or for detection of mutations (*120*).

Physical map. A map based on the chromosomal position of physical markers in units of physical distance [base pairs (bp) or kilobases (kb)].

Contig is a series of contiguous, cloned DNA fragments, assembled by determination of the overlap regions among clones (*136*). Three vectors are in current use and the size of the inserts they readily carry are as follows: YAC (yeast artificial chromosome): over 1 megabase (*103*); P1: about 100 kb (*119*); and cosmid (for plasmid with cos sites): about 40 kb.

STS (sequence-tagged site) is a short, single-copy DNA sequence that characterizes a mapping landmark in the genome and that can be detected by PCR (*109, 137*).

PFGE (pulsed-field gel electrophoresis) enable separation of large DNA fragments (200 to 3000 kb) for long-range physical mapping (*108*).

SINEs (short interspersed elements) are dispersed repetitive DNA sequences hundreds of base pairs in length. The B1 SINE is ~130 to 150 bp in length and repeated 130,000 to 180,000 times, thus making up 0.7 to 1% of the genome. B1 has strong homology to human Alu sequences. B2 is ~190 bp in length and has no known human homolog. Because of high copy number and distribution, as well as species specificity, these elements can be useful in "fingerprinting" YACs for building contigs (*138*).

LINEs (long interspersed elements) are dispersed repetitive DNA sequences (6 to 7 kb in length with 10^4 to 10^5 copies) (*138*). The LINE-1 or L1 is the major known mouse LINE and can be used to discriminate DNA from different species of *Mus* (*139*) or for "fingerprinting" YACs.

Synteny. This refers to genes on the same chromosome. Synteny conservation is defined as the occurrence of two or more pairs of homologous markers on the same chromosome in two or more species. Linkage conservation is conservation not only of synteny but also of gene order (*140, 141*).

Positional cloning. The process of gene identification, using map position as a starting point. Once the chromosomal region containing the gene is identified, DNA from that region is cloned and the gene is identified from the clones (*101*).

Candidate gene approach. The testing of cloned genes that map in the region of a mutant for involvement in the phenotype associated with a mutation (*101*).

QTL (quantitative trait loci). The set of genes that together govern the quantity of a characteristic not completely determined by any one gene acting alone (*142*).

Table 1. Effects of gene targeting on behavior or synaptic plasticity. A number of genes involved in either neuronal signaling or function have been examined for their effects on spatial learning and LTP. ND, not determined; NA, not applicable; PPF, paired-pulse facilitation; LTP, long-term potentiation; LTP(pri), primed LTP; and LTD, long-term depression.

Gene (protein)	Type of behavior	Effect of gene disruption					Reference
		Behavior	PPF	LTP	LTP(pri)	LTD	
Prn-p (PrP^c or prion)	Spatial learning	Normal	ND	ND	ND	ND	(*50*)
Camk2a (α-CaMKII)	Spatial learning	Deficit	Reduced	Deficit	Deficit*	Deficit*	(*30, 31*)
fyn (Fyn)	Spatial learning	Deficit	Normal	Deficit	ND	ND	(*32*)
src, yes, abl	ND	ND	Normal	Normal	ND	ND	(*32*)
Pkcc (PKC-γ)	Spatial, contextual learning	Mild deficit	Small increase	Deficit	Normal	Normal	(*43, 44*)
Syn-1 (synapsin I)	ND	ND	Increase	Normal	ND	ND	(*47*)
Ncam (N-CAM)	Spatial learning	Deficit	ND	ND	ND	ND	(*45*)
Nos-2 (neuronal nitric oxide synthase)	ND	ND	Normal	Small depression	ND	Normal	(*49*)
fyn	Suckling behavior	Deficit	NA	NA	NA	NA	(*58*)

*Cited in (*44*).

shows that disruption of the gene encoding PKC-γ does not eliminate, but rather modulates, LTP. The effects of PKC-γ knockouts on spatial learning are mild and are correlated with mild effects on synaptic plasticity (*44*). That α-CaMKII disruption is more severe than that of PKC-γ is clearly seen when spatial learning, LTP, primed LTP, and LTD are compared in mice with similar genetic backgrounds (Table 1): α-CaMKII mutants are deficient in all four measures, whereas PKC-γ mutants are deficient only in conventional LTP (*44*). Finally, in addition to null mutations of protein kinases, spatial learning is impaired in neuronal cell adhesion molecule (N-CAM) knockout mice (*45*), which is consistent with a role for these molecules in long-term sensitization in the marine mollusk *Aplysia* (*46*).

A number of other neuronally expressed genes that function in signaling have also been examined for participation in either behavior or synaptic transmission (Table 1), and for the most part the effects are modest. For example, knockouts of synapsin I (*47*), which is a major phosphoprotein associated with synaptic vesicles (*48*), and neuronal nitric oxide synthase (NOS) (*49*), which is implicated in LTP because of the potential role of NO as a retrograde messenger in LTP (*40, 49*), have surprisingly small effects. The NOS knockout is noteworthy because NOS inhibitors still block LTP in these mutant mice, which suggests the presence of another isoform of NOS (*49*). Indeed, the endothelial isoform of NOS is highly enriched in pyramidal cells of the hippocampus and may be the target of NOS inhibitors that block LTP (*49*). Mice with gene disruption of the prion protein (which is enriched in the nervous system and in the hippocampus) were the first knockouts to be extensively analyzed at the behavioral level, and no behavioral deficits were found, which was unexpected because of the severe neurodegenerative disorders associated with prion diseases (*50*). In addition, other knockouts of genes implicated in LTP have been created; however, these mutations are either lethal, as in the cases of the NMDA receptor NMDAR1 (*51*) and the immediate early gene c-*jun* (*52, 53*), or have not yet been analyzed for LTP or learning, as in the cases of c-*fos* (*54, 55*) and tissue-plasminogen activator (*56, 57*). Finally, in the *fyn* knockout a different behavior, the suckling of neonates, appears to be abnormal (*58*).

There are a number of important caveats in interpreting the effects of mutations. Developmental effects must be dissociated from the assumed primary effects of the mutation. For example, in *fyn* knockout mice there is a clear defect in the arrangement of granule cells in the dentate gyrus and in the pyramidal cells of the CA3 region of the hippocampus (*32*). Whether the effects of *fyn* on LTP and spatial learning are primary or secondary to its developmental effects on hippocampal anatomy remain to be established. On the other hand, compensatory mechanisms may mask the magnitude of the gene's action under normal conditions. For example, up-regulation of other nonreceptor tyrosine kinases, in the case of Fyn, or of other isoforms of CaM kinases, in the case of α-CaMKII, could ameliorate the full effects of null mutations of the genes in question. Conditional gene knockouts (which could be induced after the animals have reached adulthood) to eliminate developmental effects, as well as anatomically region-specific knockouts (which could define the critical focus for expression of mutations), should help clarify such complications in the future (*59*). Finally, an important variable for behavioral analyses concerns the genetic backgrounds in which the mutations are studied. Distinct differences in many types of behavioral measures can be seen among different inbred strains of mice (*8, 60*). Unfortunately, the 129 inbred mouse strain used for embryonic stem (ES) cells to produce knockouts is not well studied behaviorally, and it is generally difficult to maintain mutant lines on a pure 129 inbred background. Thus, the majority of knock-

Table 2. Effective germline mutagens in mice. Adapted from Rinchik (*18*).

Agent	Target	Mutation rate per locus (10^{-5})	Predominant mutation	Reference
X-rays	Spermatogonia	13 to 50	Small deletions	(*155–157*)
	Postmeiotic germ cells	33	Deletions, translocations	
	Oocytes	19	Deletions, translocations	
ENU	Spermatogonia	66 to 150	Intragenic point mutations	(*15, 68*)
Chlorambucil	Postmeiotic germ cells	127	Deletions, translocations	(*66, 67*)
Transgene	Pronucleus	~1	Insertions, deletions	(*69, 158, 159*)
Gene trap	ES cells	~10	Insertions	(*69, 160, 161*)
None		0.5 to 1.0		(*66*)

out mice are usually hybrids of 129 mice with either C57BL/6 or BALB/c mice. Because characterization of knockout mice may occur during the F_2 and F_3 generations, it is important to keep in mind that the genetic backgrounds will be segregating in these crosses and could modify effects of the mutations under study. [In both the *fyn* and PKC-γ knockouts, at least two different genetic backgrounds were assessed and the results were comparable (*32, 44*).]

Forward Genetic Approaches to Behavior in Mice

In many situations, one is faced with a phenomenon, such as behavior, in which genetic control is apparent but reverse genetic approaches are either not possible or unlikely to succeed because the genes involved are either unknown or not cloned. In such situations, classical forward genetics has been a powerful and productive approach. Mutant screens make no assumptions concerning the mechanisms underlying a behavior and require only a clear phenotype to be expressed. With efficient mutagenesis coupled with carefully crafted screening procedures, informative mutants have been isolated in many systems. For example, in the case of the cell cycle, a large number of mutants that arrest or modify the cell cycle have been isolated in yeast (*61, 62*). A surprisingly large proportion (perhaps one-third) of these mutants have defined either novel genes or critical regulatory steps within the cell cycle. Benzer and colleagues pioneered this forward genetic approach to behavior in *Drosophila* over 25 years ago (*1, 2*).

It is generally assumed that forward genetic approaches that use classical mutagenesis and screening procedures are not feasible in the mouse: "limitations imposed by attainable mutation rates, the sizes of available mouse colonies and the generation time of the mouse make it impractical to isolate mutations of a desired class" (*63*, p. 70). Although substantial investments, mutagenesis screens can be performed in the mouse successfully (*18, 64*). Table 2 compares the best known methods for producing germline mutations in the mouse. Irradiation with x-rays (*16, 65*) and treatment with chlorambucil (*66, 67*) are best for producing large deletions; however, in practice it is difficult to recover large numbers of mutant animals. The alkylating agent *N*-ethyl-*N*-nitrosourea (ENU) is best for producing point mutations, and the development of high-efficiency germline mutagenesis procedures by Russell *et al.* (*15, 68*) have made it feasible to undertake large-scale mutant screens in the mouse. Because ENU induces intragenic point mutations, it is likely that both gain-of-function and loss-of-function mutations can be produced. Finally, two methods, transgene and gene trap insertions, have the advantage of producing "tagged" mutations, but the low mutation frequencies make them unsuitable for mutant screens (*69*).

Two considerations have been critical in the production of mutations in the mouse: the achievable mutation rate and whether the germline target is premeiotic or postmeiotic. The procedures for ENU mutagenesis and screening of either dominant or recessive mutations are shown in Fig. 1. With ENU, premeiotic germ cells (spermatogonia) are the targets, and this confers two advantages. First, the G_1 progeny are nonmosaic, which is not necessarily the case with mutagenesis of postmeiotic germ cells. Second, a single mutagenized male mouse can produce a large number (100 to 150) of progeny, each of which represents one mutagenized (heterozygous) gamete. Average forward mutation frequencies of 0.0015 per locus per gamete can be achieved in the mouse with ENU (Table 2) (*68*). This means that one has a 50% chance of finding a mutation, on average, in any single locus

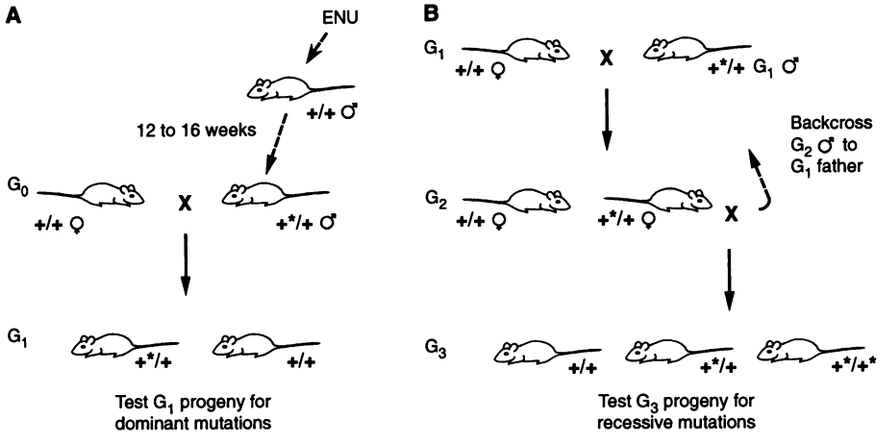

Fig. 1. Mutagenesis screen. A behavioral test can be used to detect mutations in either first- or third-generation offspring of *N*-ethyl-*N*-nitrosourea (ENU)–treated males. (**A**) Genetic screen for dominant or semidominant mutations in first-generation (G_1) offspring. Male mice were injected intraperitoneally with ENU to intervene at the stage of spermatogonia and enter a period of sterility. Upon recovery of fertility (12 to 16 weeks after treatment), they were bred with multiple females to produce G_1 progeny that were heterozygous for any ENU-induced mutation (indicated by an asterisk). (**B**) Genetic screen for recessive mutations in third-generation (G_3) offspring. In this scheme, G_1 male progeny are bred with wild-type females to produce second-generation (G_2) progeny. The G_2 female progeny are then bred with their G_1 father to produce G_3 progeny. Compared to intercrossing G_2 females and males, backcrossing G_2 females to the G_1 father doubles the likelihood of producing G_3 progeny that are homozygous for a mutation.

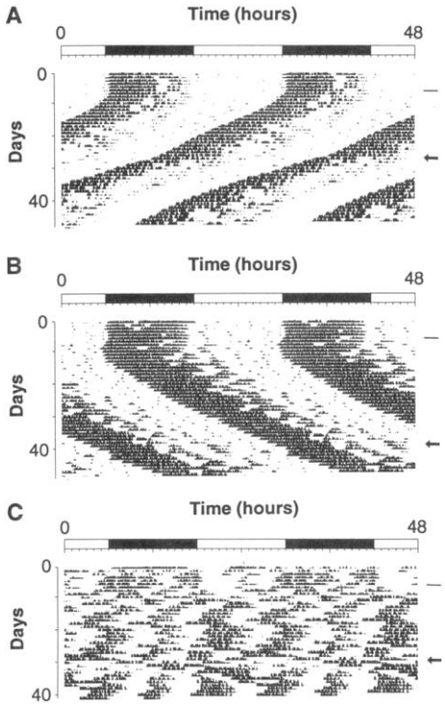

Fig. 2. Locomotor activity records of *Clock* mutant mice. The wheel-running activity records of three (BALB/cJ × C57BL/6J)F_2 offspring are shown. All animals were kept on a light-dark cycle of 12 hours (LD 12:12) for the first 7 days illustrated, then transferred to constant darkness (DD) on the day indicated (line on the right); they later received a 6-hour light pulse on the day indicated (arrow). (**A**) Activity record of a wild-type F_2 mouse. In DD, this animal's activity rhythm had a period of 23.1 hours. (**B**) Activity record of a heterozygous *Clock*/+ F_2 mouse. In DD, this animal's activity rhythm had a period of 24.9 hours. (**C**) Activity record of a homozygous *Clock/Clock* F_2 mouse. This individual had a complete loss of circadian rhythmicity upon transfer to DD, with a rhythm of 28.4 hours transiently expressed after the light pulse.

by screening about 655 gametes. In the case of a dominant screen, each G_1 mouse represents one mutagenized gamete (Fig. 1A). To approach saturation mutagenesis (that is, to be able to mutate all loci at least once assuming Poisson statistics and a mutation frequency of 0.001 per locus per gamete), one would have to screen about 3000 gametes. In contrast, to screen for recessive mutations, each male G_1 mouse is used to found a three-generation pedigree in which three G_2 females are backcrossed to the G_1 parent in order to isolate G_3 progeny that are homozygous for the mutagenized gamete (Fig. 1B). To insure an 80% "efficiency of scanning" of the genome, six progeny from each of the three daughters must be tested in each pedigree (*70*).

Although this procedure is daunting, Shedlovsky, Dove, and colleagues have successfully performed recessive screens of about 350 gametes each to isolate mutations in the *t* region (*70*) and in the phenylalanine hydroxylase (*Pah*) locus (*71*). Two additional alleles at the *Pah* locus were then obtained in a locus-specific noncomplementation screen of 350 mutagenized gametes (*21*). Finally, saturation mutagenesis screens have been performed in two regions of the mouse genome: the *t* region of mouse chromosome 17 already mentioned (*20*) and the *albino* (*c*) region of chromosome 7 with a hemizygous screen over a large deletion (*19*). Thus, even when one must make mutagenized gametes homozygous for the screening of recessives, the extremely high efficiency of ENU mutagenesis makes it possible to isolate mutants of a desired class. At a rate of 300 mutagenized gametes screened per year, it would take about ten laboratory years to reach saturation of the mouse genome. Thus, one could imagine two laboratories completing a saturation screen in 5 years.

Until recently, mutagenesis screens with behavioral endpoints have not been undertaken in the mouse. Three factors are critical in considering the feasibility of behavioral genetic screens. First, the behavioral screen should either be easy to perform or be easy to automate so that the phenotypes of large numbers of mice can be determined. A starting point would be determining the phenotypes of 1000 animals per year in a dominant screen. Second, the ability to determine the phenotype of individual animals is extremely useful (and perhaps essential) in genetic mapping experiments. Finally, the possibility of screening for dominant mutations should be taken seriously because only in this case will it be feasible to screen enough mice to approach genome-wide saturation mutagenesis (of genes capable of yielding dominant mutations). Given the previous calculations, a saturation screen (3000 mutagenized gametes) for dominant mutations with a behavioral assay could be achieved in 3 years.

With these considerations in mind, we initiated a behavioral screen for ENU-induced mutations of the circadian system in the mouse (*72*). Circadian rhythms are 24-hour oscillations in an organism's behavior and physiology that are the overt manifestation of an internal physiological clock system (*73, 74*). In mammals, much is known about the physiology of circadian rhythms, and the suprachiasmatic nucleus of the hypothalamus acts as a circadian pacemaker controlling circadian rhythms expressed at the organismal level (*75, 76*). On the basis of a wide variety of evidence, the mechanism of the circadian clock appears to be cell-autonomous and to involve periodic gene expression (*74*). In *Drosophila* and *Neurospora*, a number of "clock mutants" have been isolated (*77–79*). Recent molecular work with the *Drosophila period* (*per*) and *Neurospora frequency* (*frq*) genes suggests that circadian cycles of *per* and *frq* transcription, respectively, which involve negative autoregulatory feedback loops, may lie at the heart of the oscillator mechanism in these species (*80–83*). However, little information exists concerning the molecular elements of the clock system in mammals.

Because circadian behavior in the mouse is precise and easily quantitated, it is especially suited for genetic screening. Normal C57BL/6J mice exhibit a robust circadian rhythm of wheel-running activity (*84, 85*), and we used this behavioral assay to screen for mutants that expressed abnormal circadian periods in constant darkness. Because

Box 2. Mouse genome resources and informatics

DATABASES

GBASE, the genomic database of the mouse, compiles published genetic mapping data and is available with a character-cell interface online over Internet.

Encyclopedia of the Mouse Genome consists of software tools that display genetic linkage maps, cytogenetic maps, and a text searching tool. Data from several independent sources are integrated, including the Mouse Chromosome Committee reports, MIT Genome Center data releases, mammalian comparative mapping data, GBASE, the Mouse Locus Catalog, and mouse cytogenetic mapping data. Data sets may be obtained with File Transfer Protocol (FTP), Gopher, or World Wide Web (WWW) software. Software is available in Sun (UNIX) and Macintosh versions.

The Mouse Genome Database (MGD) integrates existing databases of mouse genetic information and will include mapping data, molecular probes-clones data, strains and allelic polymorphism data, phenotypic information, and comparative mapping data. Initial availability of the database will be through WWW beginning the third week of June 1994.
For any of the above, contact: Mouse Genome Informatics-User Support, The Jackson Laboratory, 600 Main Street, Bar Harbor, ME 04609, USA. Phone: 207 288 3371 ext 1900; fax: 207 288 2516; Internet: mgi-help@informatics.jax.org

Whitehead Institute/MIT Genetic Map of the Mouse consists of SSLPs mapped on a (C57BL/6J-*ob*/*ob* × CAST)F_2 cross (*23–25*). The newest release has information about 3752 SSLPs and also integrates data from a second interspecific backcross panel, which is gene-based and developed at the National Cancer Institute Frederick Cancer Research and Development Center, Frederick, MD. The database includes PCR primer pair sequences, genotypes, product allele sizes for multiple inbred strains and MacDraw maps of chromosomes. Lists of markers can be retrieved by email and selected for polymorphism between pairs of strains or for chromosome, map position, or name. *Contact:* Eric Lander, Whitehead Institute/MIT Center for Genome Research, Cambridge, MA 02142, USA. Phone: 617 252 1900; fax: 617 252 1933; Internet: lander@genome.wi.mit.edu
To receive a query form and instructions, send email message "help" to: genome_database@genome.wi.mit.edu

The Portable Dictionary of the Mouse Genome is a compact database containing information on 12,000 genes and anonymous DNA loci in the mouse (*143*). The dictionary includes three separate estimates of gene position, accession numbers to GenBank sequences, data on homologs in human and 10 other mammalian species, a complete set of data on recombinant inbred strain distribution patterns, data on phenotypes, PCR primers, alleles, references, and several additional data types. The 10-megabyte dictionary file is designed for use on either Macintosh or PC. It is available in FileMaker Pro, Excel, and text formats and can be easily converted for a variety of other applications. The entire dictionary or chromosome-specific files are available via Internet (WWW, Gopher, or FTP) The dictionary is available on CD-ROM (National Center for Biotechnology Information) or on floppy disk (R. Williams).
Contact: Robert W. Williams, Center for Neuroscience, University of Ten-

most clock mutations isolated in other organisms have been semidominant (*7, 79*), we performed a dominant screen with the procedure shown in Fig. 1A. In testing about 300 gametes of ENU-treated mice, one animal expressed a circadian period that was more than 1 hour longer than normal. The long-period phenotype was inherited as a semidominant autosomal mutation, which we named *Clock* (*72*) (Fig. 2). Homozygous *Clock* mice expressed extremely long periods of 27 to 28 hours upon initial transfer to constant darkness, which was followed by a complete loss of circadian rhythmicity after about 2 weeks in constant darkness (Fig. 2C). The *Clock* gene thus regulates at least two fundamental properties of the circadian clock system: the intrinsic circadian period and the persistence of circadian rhythmicity. Because a wild-type allele of *Clock* is necessary for sustained circadian rhythmicity, *Clock* defines an essential gene for this behavior. Moreover, because no anatomical or developmental defects have been observed in association with the *Clock* mutation (*72*), *Clock* appears to be a "behavioral mutation" limited to circadian rhythmicity.

Given the extensive genetic mapping information available in the mouse (Box 2), we were able to map *Clock* rapidly by linkage analysis using intraspecific mapping crosses and simple sequence length polymorphisms (SSLPs) from the Whitehead Institute/MIT genetic map (*72*). *Clock* mapped to the midportion of mouse chromosome 5 between two SSLP markers, *D5Mit24* and *D5Mit83*, in a region that shows conserved synteny with human chromosome 4 (*25*).

Forward genetics may be one of the few ways in which to identify genes involved in the clock mechanism in mammals. Because the two examples of cloned clock genes (*Drosophila per* and *Neurospora frq*) are unique and are expressed in low abundance (*78, 79*), it is unlikely that these genes could have been identified and their function studied by other means. It is also important to emphasize that in both *Drosophila* and mice, other genetic approaches such as selection of natural variants, comparison of strain differences, or recombinant inbred (RI) strain analysis all initially suggested genetic control but yielded subtle differences that were polygenic and "unmappable" (*78, 85–87*). These results suggest that natural allelic variation is normally not as extreme as that attainable by induced mutagenesis, because in both *Drosophila* and mice single genes that strongly influence circadian rhythmicity have been identified by forward genetics. Whether this difference will generally apply to other types of behavior should be kept in mind.

Despite the lack of success with the use of quantitative genetics in identifying genes regulating circadian behavior, this approach should not be abandoned. With high-density genetic maps in the mouse, techniques are available to map quantitative trait loci (QTL), making it possible to identify multiple genes involved in differences between strains or between lines selected for a behavioral trait. QTL analysis, which was pioneered in plant genetics (*88*), has now been successfully applied to the mapping of genes involved in autoimmune type 1 diabetes in nonobese diabetic (NOD) mice (*89*) and Brattleboro (BB) rats (*90*), hypertension in stroke-prone spontaneously hypertensive rats (*91*), epilepsy in different strains of mice (*92*), and audiogenic seizures in RI strains (*93*). A particularly important variant of the QTL approach was recently used to map a modifier gene, *Mom-1*, that influences the quantitative expression in different genetic strain backgrounds of a single-gene mutation, *Min*, that leads to the formation of intestinal tumors (*94*). This example clearly shows the advantage of combining single-gene approaches with QTL analysis to identify interacting loci that could not be detected by use of natural variants. Single-gene behavioral mutants could be used in an identical fashion to "sensitize" and detect other interacting genes that influence the expression of a behavioral trait in different genetic backgrounds.

Another consideration in the use of forward genetics concerns the feasibility of approaching saturation mutagenesis. This is important for two reasons. First, one would

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HUGO Mouse Genome Resources Database provides an international listing on mouse genome mapping resources and mouse genome mapping projects that are not generally available through established outlets. The database is compiled on Macintosh *Hypercard*, available on disk, and is updated every 6 months.
To receive a disk copy of the database, contact:
HUGO Europe, One Park Square West, London NW1 4IJ, UK.
Phone: 44 71 935 8085; fax: 44 71 935 8341
For information, contact: Steve Brown, Department of Biochemistry and Molecular Genetics, St. Mary's Hospital, London W2 1PG, UK.
Phone: 44 71 723 1252 ext 5484; fax: 44 71 706 3272; Internet: s.brown@sm.ic.ac.uk

Reference Library Database (RLDB). Cosmid, YAC, P1, and cDNA libraries are distributed on high-density filters to the scientific community and experimental results are stored in a common object-based database accessible through Internet, dial-up service, or WWW (*112*).
Contact: Reference Library Database, Imperial Cancer Research Fund, Room A13, 44 Lincoln's Inn Fields, London WC2A 3PX, UK.
Phone (G. Zehetner): 44 71 269 3571; fax: 44 71 269 3479; Internet: genome@icrf.icnet.uk

TBASE is a transgenic animal–targeted mutation database organized by Oak Ridge National Laboratory and Johns Hopkins University, available through Internet (Gopher) or Telnet (*144*).
Contact (data additions): Karin Schneider, ORNL TBASE Coordinator, Human Genome and Toxicology Group, Oak Ridge National Laboratory, 1060 Commerce Park, MS-6480, Oak Ridge, TN 37831, USA.
Phone: 615 574 7776; fax: 615 574 9888; Internet: tbase@iravx2.hsr.ornl.gov
Contact (electronic access): Dan Jacobson or Anna Anagnostopoulos, Laboratory for Applied Bioinformatics, Johns Hopkins University, 2024 East Monument Street, Baltimore, MD 21205, USA.
Phone: 410 955 1704 (D.J.) or 410 614 3226 (A.A.); fax: 410 614 0434; Internet: danj@gdb.org or anna@gdb.org or help@gdb.org

Entrez provides nucleotide and protein sequence information, MEDLINE citations in which the sequences were published, and a sequence-associated subset of MEDLINE (*145*). The sequence records are derived from a variety of database sources, including GenBank, EMBL (European Molecular Biology Laboratory Data Library), DDBJ (DNA Data Bank of Japan), PIR (National Biomedical Research Foundation, Martinssried Institute for Protein Sequences, and Japan Institute for Protein Information Database), SWISS-PROT (Amos Bairoch and EMBL Protein Sequence Database), PRF (Protein Research Foundation, Osaka), and PDB (Brookhaven Protein Data Bank). The retrieval software and associated databases are distributed on CD-ROMs, updated bi-monthly. These are distributed by subscription through the U.S. Government Printing Office. The software is public domain, available in Macintosh or PC (Windows) format. In addition, ***Network Entrez*** is now available by Internet.
Contact: National Center for Biotechnology Information, National Library of Medicine, NIH, Building 38A, 8600 Rockville Pike, Bethesda, MD 20894, USA.
New orders: phone: 202 783 3238; fax: 202 512 2233
Order status queries: phone: 202 783 3238; fax: 202 512 2168
General questions (Internet): info@ncbi.nlm.nih.gov
Network Entrez questions (Internet): net-info@ncbi.nlm.nih.gov

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like to identify and define all of the genes that are essential for a behavior in order to have some measure of the complexity of the system: Will there be hundreds of genes involved or will only a handful be critical? Such assessments have been achieved only in organisms such as *Drosophila* for genes regulating pattern formation (*95*) and are on the horizon for zebrafish (*96, 97*). One can imagine that this goal is attainable for circadian behavior in *Drosophila* and perhaps in the mouse for genes mutable to a dominant phenotype. Second, the isolation of multiple mutations is crucial because many are likely to be uninformative—being either nonspecific, pleiotropic, or secondary to a developmental effect. Because the recovery of such uninformative mutants is unavoidable, the only ways to insure that informative mutants will be found are to use stringent phenotyping methods and to test large numbers of mutagenized gametes.

Finally, the isolation of mutations is not only useful in identifying genes, but also provides advantages for physiological analysis. For example, mosaic analysis, in which animals express a patchwork of either mutant or wild-type cells, can be used to identify the critical tissue "focus" of a gene's action. In *Drosophila*, mosaic analysis with the clock gene *per* has localized cells in the brain required for robust expression of circadian behavioral rhythms (*98*). In mammals, the *tau* mutation in the hamster, which shortens circadian period by 2 hours in heterozygotes and by 4 hours in homozygotes (*99*), has been used in transplantation experiments to show that expression of *tau* in the suprachiasmatic nucleus region is sufficient for regulation of circadian behavior and that the suprachiasmatic nucleus contains a circadian pacemaker that controls period in hamsters (*100*). Thus, mutations enable both transplantation and mosaic analysis to be applied to the study of behavior.

Mouse molecular genetics will likely be crucial to unraveling the mechanisms underlying behavior in mammals. The power of ENU mutagenesis combined with the ability to clone genes by map position provides a way to study complex behavior in mammals. If efficient screening procedures can be devised, this approach should have widespread utility in neuroscience and behavior to analyze processes such as learning and memory.

Positional Cloning of Mutations in Mice

Given the substantial genetic and physical mapping resources being applied to studies of the mouse (Box 2), it should be feasible to clone almost any mutation. As reviewed by Copeland *et al.* (*25*), about 40 classical mouse mutations have been cloned. Three-quarters of these were cloned by the "candidate gene" approach, which involves mapping a new cloned gene and determining whether any existing mutants mapping in the region have phenotypes that are consistent with an alteration in the cloned gene. The candidate gene is then tested by comparing wild-type and mutant mice for mutations at the molecular level. If no obvious candidate genes exist in the region of the mutation, the strategy for cloning a gene defined solely by a mutant phenotype is to use the method of positional cloning (*101*). This method requires a high-resolution meiotic (genetic) map position derived from crosses segregating the mutation of interest before initiating physical mapping and cloning procedures. Once an initial chromosomal map position for the mutation is established, an expanded backcross with 1000 to 2000 meioses must be produced to have sufficient resolution to proceed with physical mapping. To optimize this fine-structure meiotic mapping step (*102*), one selects flanking markers within a few centimorgans (cM) of the mutation, and only animals with recombination between flanking markers are assayed for phenotype. Screening 1000 meioses will yield a map position within a 0.3-cM interval (at 95% probability), which is equivalent in physical units to the insert size (~600 kb) of available yeast artificial chromosome (YAC) libraries (*103, 104*) in the mouse (*105–107*). Next, estimates of physical linkage of the genetic markers in the fine-structure meiotic mapping should be determined by long-range restriction mapping and pulsed-field gel electrophoresis with markers converted to single-copy probes

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GENETIC MAPPING RESOURCES

Interspecific crosses have been particularly valuable in mapping mouse genes because of the high degree of polymorphism (*146*). Five different groups have created interspecific crosses for mapping with cloned DNA probes: (i) Guénet and colleagues (*146*); (ii) Jenkins and Copeland and colleagues (*25*); (iii) Seldin and colleagues (*147*); (iv) Kozak and colleagues (*148, 149*); and (v) Lander and colleagues (*23, 24*). In addition, any gene that has been cloned can be mapped with DNA probes using the publicly available DNA panels listed below. However, any gene defined by a phenotype (such as a newly identified mutation) must be mapped by establishing a new cross in which the mutation segregates.

The Jackson Laboratory Backcross DNA Panel Map Service maintains and distributes DNA from two reciprocal interspecific (C57BL/6J × *M. spretus*) backcrosses (94 progeny in each) as well as two parental DNAs (*150*). The DNAs have been characterized for MIT SSLP markers, proviral loci, and several other sequence-defined genes. Aliquots of DNA for either Southern blot analysis or PCR templates are available, as well as limited Southern blot filter sets. The public data are available from MGD or the Portable Database of the Mouse Genome.
Contact: Lucy Rowe or Mary Barter, Jackson Laboratory, 600 Main Street, Bar Harbor, ME 04609, USA.
Phone: 207 288 3371 ext 1687; fax: 207 288 5079; Internet: lbr@aretha.jax.org (L.R.) or meb@aretha.jax.org (M.B.)

European Collaborative Interspecies Backcross (EUCIB). A total of 982 progeny from reciprocal interspecific (C57BL/6J × *M. spretus*) backcrosses have been scored for at least three markers for each chromosome (*151*). DNA from individuals recombinant between any two anchor markers are available upon request for PCR or Southern blot analysis, enabling high-resolution mapping of a region of interest.
Contact: Steve Brown, Department of Biochemistry and Molecular Genetics, St. Mary's Hospital, London W2 1PG, UK.
Phone: 44 71 723 1252 ext. 5484; fax: 44 71 706 3272; Internet: s.brown@sm.ic.ac.uk

Recombinant inbred (RI) strain panels can be used to map allelic differences between common laboratory inbred strains. A number of RI strains were established by Taylor and others at the Jackson Laboratory and the strain distribution pattern of many markers are described (*131, 152*). Map resolution is limited by the number of strains in the panel. At least 13 panels, with different parental inbred strain combinations and consisting of at least five strains, are available from the Jackson Laboratory. Databases of genes mapped in RI strains are available in both the Portable Database of the Mouse Genome and Map Manager.
Contact: Jackson Laboratory Animal Resources, 600 Main Street, Bar Harbor, ME 04609, USA.
Phone:1 800 422 MICE or 207 288 3371; fax: 207 288 3398

SOFTWARE

MAPMAKER/EXP 3.0 is a linkage analysis package for constructing primary linkage maps (*153*). It can do multipoint linkage analysis for markers in backcross and intercross panels as well as RI lines.

MAPMAKER/QTL 1.1 is the companion program that allows one to map genes controlling polygenic quantitative traits in intercross and backcross progeny relative to a genetic linkage map (*142*). Both programs are avail-

(*108*). At the same time, screening of mouse YAC libraries can begin either with sequence-tagged sites (STSs) (*109*) (Box 1) [with use of the polymerase chain reaction (PCR) of linked SSLP markers and cloned DNA sequences (*110*)] or with arrayed YAC clones on filters [with use of cloned DNA probes (*111*)] to build a contiguous segment of cloned genomic DNA with overlapping YAC clones that covers the region of the mutation; this set of overlapping clones is called a "contig" (Box 1). This step has been facilitated recently by the commercial availability of a C57BL/6J mouse YAC library that is already pooled and ready for PCR analysis (*107*) and by the availability of YAC clones arrayed on filters (Box 2) (*112*).

If a reasonable number of YAC clones covers the region of the mutation and the mutant can be rescued by wild-type DNA, then one can contemplate expressing candidate YAC clones in mouse to complement or "rescue" the mutation. This technical feat has been accomplished with germline transmission and expression of YAC clones in the mouse either by pronuclear injection of gel-purified YAC DNA with transgenic methods (*113*) or by introducing purified YAC DNA into ES cells by lipofection and producing chimeric mice that transmitted the YAC transgene through the germline (*114*). Once a YAC clone has been found that rescues the mutant, then all effort can be focused on such clones to identify transcription units and determine which gene encodes the mutation within the YAC clone. Currently, the most efficient method for identification of expressed sequences appears to be "exon amplification" (*115–118*) of cosmid or P1 clones (*119*) derived from the YAC. Finally, mutations in individual candidate genes can be identified with single-strand conformation polymorphism (SSCP) (*120*) or other methods (*121*). The final proof that the gene has been identified is confirmed by expression of the gene and rescue of the mutant phenotype in transgenic mice.

Positional cloning of mutations can be an arduous task, but at least four mutations, *Brachyury* (*T*) (*122*), *short ear* (*se*) (*123*), *agouti* (*a*) (*124, 125*), and a mycobacterial resistance gene (*Bcg*) (*126*), have been cloned by this method in mice. In the case of the *Brachyury* and *short ear* genes, a large number of deletions were available to facilitate physical mapping of the region. A radiation-induced inversion mutation with a breakpoint within the *agouti* locus was instrumental in cloning this gene. So far, only the *Bcg* gene has been cloned in the mouse solely by its map position with the use of "pure" genetics.

In the near future, positional cloning methods will become even more powerful. The Whitehead Institute/MIT genetic map will be completed with a total of 6000 SSLPs by 1995. In addition, these 6000 SSLPs will be mapped on the European Collaborative Interspecies Backcross (EUCIB) panel that has 1000 meioses and 0.3-cM resolution (Box 2). Furthermore, a complete physical map of the mouse genome in the form of STS-ordered YAC clones should be complete in about 3 to 5 years (*26, 127*). Ultimately, the human and mouse genomes are planned to be sequenced side by side (*127*) so that functional analysis rather than cloning will become the rate-limiting step in analyzing the genetics of behavior.

Summary

The single-gene approach can be successfully applied to study behavior in the mouse with the use of both forward and reverse genetics. Both approaches have only recently been applied to the analysis of the mechanisms underlying behavior. Gene targeting provides a novel approach to study the functional role of identified genes in regulating behavior. Because it is clear that the genes for a large number of key molecules involved in neuronal signaling are targets for disruption in many laboratories, there will be no shortage of mutations to examine as candidates for behavioral analysis in the future. In a complementary manner, ENU mutagenesis and screening for behavioral mutants provides an efficient approach to identify previously unknown genes with the use of phenotype-driven methods. With behavior, this forward ge-

able in Sun (UNIX), PC (DOS), and Macintosh versions.
Contact: MAPMAKER c/o Eric Lander, Whitehead Institute, 9 Cambridge Center, Cambridge, MA 02142, USA.
Fax: 617 258 6505; Internet: mapmaker@genome.wi.mit.edu

Map Manager v 2.5 is a specialized database program designed to help store, organize, and analyze the results of genetic mapping experiments using backcrosses, intercrosses, or RI strains (*154*). It includes a database from RI strains and is available for Macintosh computers.
Contact: Kenneth F. Manly, Rosewell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY 14263, USA. Phone: 716 845 3372; fax: 716 845 8169; Internet: kmanly@mcbio.med.buffalo.edu

PUBLICATIONS AND BULLETIN BOARDS

Mouse Genome is published quarterly by Oxford University Press. It provides updates on mouse mutants, variants, linkages, and inbred strains.
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Mammalian Genome is the monthly official publication of the International Mammalian Genome Society. A subscription is included in membership fees. A supplement publishes reports from the mouse chromosome committees annually.
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Human Genome News is published monthly, sponsored by the National Institutes of Health and the U.S. Department of Energy. It provides a variety of information on new resources, meetings, and news relevant to the Human Genome Project.
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Phone: 410 955 1704; fax: 410 614 0434; Internet: danj@gdb.org

Mouse Genome Informatics Group at the Jackson Laboratory has set up an electronic bulletin board with email addresses of mouse genetics community members registered in the group's user support database. The bulletin board operates by automatically forwarding email messages it receives to all its known subscribers. The Mouse Genome Informatics Group will make announcements of new software and data sets through this MGI-LIST Bulletin Board. Special bulletin boards for each of the mouse chromosome committees have been set up to facilitate their interactions. In the future, additional bulletin boards can be established as needed, and announcements of their creation will be made through MGI-LIST. *Contact:* Mouse Genome Informatics-User Support, Jackson Laboratory, 600 Main Street, Bar Harbor, ME 04609, USA.
Phone: 207 288 3371 ext 1900; fax: 207 288 2516; Internet: mgi-help@informatics.jax.org
To subscribe, send email message "subscribe mgi-list <your name>" to: listserver@informatics.jax.org

netic approach could be especially powerful because in most cases the underlying mechanisms are unknown. Coupled together with the genetic and physical mapping resources available from the "new mouse genomics" (Box 2), mutations defined by phenotype alone can be molecularly identified by the method of positional cloning. Thus, mouse genetics has entered a new era: it is now possible to study the "genomics of behavior."

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162. Special thanks are due to W. F. Dove, whose wisdom and influence are reflected throughout this paper. We thank E. R. Kandel, S. Tonegawa, E. S. Lander, S. Brown, N. A. Jenkins, N. G. Copeland, K. Manly, M. Barter, L. Rowe, J. Eppig, and R. W. Williams for advice and comments; and our colleagues in the NSF Center for Biological Timing for their support. Supported by a grant from the MacArthur Foundation Network on Depression, the NSF Center for Biological Timing, a State of Illinois HECA award (J.S.T., F. W. Turek, and L.H.P.), NIH grants MH39592, MH49241, and EY08467 (J.S.T.), and postdoctoral training awards T32 NS07140 and T32 DC00015 (M.H.V.).

The Genetic Basis of Complex Human Behaviors

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Quantitative genetic research has built a strong case for the importance of genetic factors in many complex behavioral disorders and dimensions in the domains of psychopathology, personality, and cognitive abilities. Quantitative genetics can also provide an empirical guide and a conceptual framework for the application of molecular genetics. The success of molecular genetics in elucidating the genetic basis of behavioral disorders has largely relied on a reductionistic one gene, one disorder (OGOD) approach in which a single gene is necessary and sufficient to develop a disorder. In contrast, a quantitative trait loci (QTL) approach involves the search for multiple genes, each of which is neither necessary nor sufficient for the development of a trait. The OGOD and QTL approaches have both advantages and disadvantages for identifying genes that affect complex human behaviors.

The received wisdom of the behavioral sciences concerning the importance of "nature" (genetics) and "nurture" (environment) in the origins of behavioral differences among people has changed dramatically during the past few decades. Environmentalism, which attributes all that we are to nurture, peaked in the 1950s. A more balanced view that considers both nature and nurture swept into psychiatry in the 1960s and 1970s. Although this balanced view has been slower to reach some realms of psychology, there are signs that it has arrived. For example, at its centennial meeting in 1992, the American Psychological Association identified genetics as one of the themes that best represent the present and especially the future of psychology (*1*).

Behavioral genetic research began in the 1920s with inbred strain and selection studies of animal behavior and family, twin, and adoption studies of human behavior (*2*). These quantitative genetic designs assess the "bottom line" of transmissible genetic effects on behavior, regardless of the number of genes involved, the complexity of their interactions, or the influence of nongenetic factors. As discussed in the first part of this article, quantitative genetic research has built a strong case for the importance of genetic factors in many complex dimensions and disorders of human behavior.

Although more quantitative genetic research is needed, the future of behavioral genetics lies in harnessing the power of molecular genetics to identify specific genes for complex behaviors. In the second part of this paper, initial successes are described and research strategies are discussed. Although more powerful methods and results are available for the investigation of animal than human behavior, animal work is discussed in accompanying articles in this issue.

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Quantitative Genetics

The change from antipathy to acceptance of genetic factors in the behavioral sciences has occurred so rapidly and thoroughly, especially in psychiatry, that a reminder is warranted about how environmentalistic the behavioral sciences were, even in the 1960s. For example, the major explanation for schizophrenia was abnormal parenting.

Adoption studies were pivotal in leading psychiatrists to consider nature as well as nurture. Schizophrenia was known to run in families, with a risk of 13% for offspring of schizophrenic parents, 13 times the population rate of about 1% (*3*). Adoption experiments allow a determination of whether schizophrenia runs in families for reasons of nature or of nurture. In a classic study, Heston (*4*) examined the offspring of schizophrenic mothers who had been adopted at birth and compared their rate of schizophrenia to a control group of adopted offspring. Of the 47 adopted-away offspring of schizophrenic mothers, 5 were diagnosed as schizophrenic, as compared to none of the 50 control adoptees. Indeed, the risk of schizophrenia for the adopted-away offspring of schizophrenic mothers is the same as the risk for individuals reared by a schizophrenic parent.

These findings implicating substantial genetic influence in schizophrenia have been replicated and extended in other adoption studies, and they confirm the results of twin studies that show greater concordance for identical twins (about 45%) than fraternal twins (about 15%) (*3*). This twin method is a natural experiment in which the phenotypic resemblance for pairs of genetically identical individuals [identical, monozygotic (MZ) twins] is compared to the resemblance for pairs of individuals whose coefficient of genetic relationship is only 0.50 [fraternal, dizygotic (DZ) twins].

The convergence of evidence from family, twin, and adoption designs—each with distinct assumptions—provides the most convincing argument for the importance of genetic factors in behavioral traits.

Behavioral disorders. Evidence for genetic influence has been found for nearly all behavioral disorders that have been investigated (*5*). Figure 1 summarizes the results