Mouse models for psychiatric disorders

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Genes involved in psychiatric disorders are difficult to identify, and those that have been proposed so far remain ambiguous. As it is unrealistic to expect the development of, say, a 'schizophrenic' or 'autistic' mouse, mice are unlikely to have the same role in gene identification in psychiatry as circling mice did in the discovery of human deafness genes. However, many psychiatric disorders are associated with intermediate phenotypes that can be modeled and studied in mice, including physiological or anatomical brain changes and behavioral traits. Mouse models help to evaluate the effect of a human candidate gene mutation on an intermediate trait, and to identify new candidate genes. Once a gene or pathway has been identified, mice are also used to study the interplay of different genes in that system.

Published online: 01 November 2002

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The mouse has been used as a genetic model organism in all fields of medicine – from limb development to diabetes and alcoholism. The advantages of mice are clear to geneticists: (1) mice are available in many different inbred strains; (2) the mouse genome sequence is almost complete; (3) mutagenesis can isolate new mutations; and (4) mice can be genetically manipulated in ways not possible in other species. Additional TRANSGENES (see Glossary) can be expressed in mice using well characterized promoters, specific mutations can be KNOCKED IN, and genes can be KNOCKED OUT not only in the complete organism, but also in a tissue-specific or developmental stage-specific manner. Less complex organisms such as yeast and *Caenorhabditis elegans* have shorter generation times and are the best models for studying simple metabolic pathways. Higher mammals such as dogs or monkeys are better for certain complex social behaviors. But these advantages pale when compared with the genetic strengths of the mouse as an easily manipulated mammal.

What can be modeled – what cannot?

For highly heterogeneous human disorders that are inherited as a simple mendelian trait in each family, mouse models have been extremely successful in identifying the genes involved. For example, there are more than 80 different human nonsyndromic deafness loci. Of these, more than 30 have been cloned, many by first identifying the defect in mouse mutants [1]. But only very few psychiatric conditions are caused by single gene defects that can be modeled in mice. One such rare example is Brunner syndrome, a severe condition found only in a single family, in which the MaoA protein is not made [2]. The aggressive behavior that is part of this syndrome could be replicated faithfully in mice, and these mice were useful in elucidating the neurochemical changes in this disorder [3]. Mice are also used extensively to model addiction to alcohol and other drugs of abuse, but this field has been reviewed elsewhere [4,5] and will not be discussed further here.

It is difficult to envision a perfect mouse model for any of the common psychiatric illnesses. Mice simply lack the higher, typically human functions of, for example, suicidality, concepts of self, self reflection, consideration of others or language that are deficient in disorders such as depression, schizophrenia, antisocial personality disorder and autism (see [6] for a review of the common psychiatric illnesses, symptoms and epidemiology). However, these disorders are associated with quantitative phenotypes called INTERMEDIATE TRAITS or ENDOPHENOTYPES (Fig. 1, Table 1). These traits are risk factors that are, in many cases, more common than the full disorder, and can be closer to the genetic etiology than the full syndrome. Examples are anxiety in depression, PRE-PULSE INHIBITION (PPI) in schizophrenia and social interaction deficits in autism and schizophrenia. In contrast to the full complex disorder, some of these traits can readily be modeled in mice (Table 1). Although we refer to some of these mice as models of a disorder in this article, they model only specific traits of the disease.

Pharmacological evidence for mouse models

When considering whether such an intermediate trait is appropriate as a model for a psychiatric disorder, additional evidence for the validity can be provided by pharmacological studies [7]. For example, although the PORSOLT FORCED SWIM TEST is not intuitively a good model for depression, it is useful to predict the antidepressant response of a drug – the most efficacious antidepressants also restore swimming in this mouse model [7]. Similarly, even though methylphenidate (Ritalin®) is a stimulant, it has a paradoxical, calming response in humans suffering attention deficit hyperactivity disorder (ADHD). By contrast, hyperactivity in pervasive developmental disorders such as autism does not respond well to stimulants. Response of a hyperactive mouse model to methylphenidate can therefore distinguish between different forms of hyperactivity [8,9]. Thus, pharmacological data can increase our confidence in the role of a specific trait as a component of a psychiatric disorder, and can distinguish between different models.

 0168-9525/02/$ – see front matter PII: S0168-9525(02)02807-X

Glossary

Acute/chronic stress in mice: One of the simplest models of acute stress in mice is physical restraint, in which mice are restrained for a brief period. Other commonly used stressors in mice are social isolation, cold, swim, noise and food restriction. The length and intensity of the stressor can be varied to induce acute/chronic or mild/severe stress.

Emotionality in mice: Defecation and locomotor activity in a novel, brightly lit open-field (an adversive environment) are used as measures of emotionality. It correlates with level of fearfulness and is thought to have some parallels with human anxiety. Low locomotor activity and high defecation scores indicate high emotionality; high activity and low defecation indicate low emotionality.

ENU mutagenesis: ENU (ethylnitrosourea; *N*-ethyl-*N*-nitrosourea), a chemical mutagen induces point mutations in DNA. Using ENU, allelic series of a gene can be generated including gain-of-function as well as complete and partial loss-of-function mutations. Male spermatagonial germ cells are the usual target of mutagenesis and offspring are screened for phenotypes of interest. After inheritance of the phenotype is confirmed, mouse lines are subjected to genetic mapping to identify the responsible mutations.

Epistatic interaction: When one gene influences the phenotypic expression of another gene.

Heterogeneous stock (HS): The heterogeneous stock was established from an eight-way cross of C57BL, BALB/c, RIII, AKR, DBA/2, I, A and C3H/2 inbred mouse strains. The large number of founders increases the genetic heterogeneity and promotes mapping quantitative trait loci of small to moderate effect. Each chromosome from an HS animal is a fine-grained genetic mosaic of the founder strains, with an average distance between recombinants of 1/60 or 1.7 cM. This high level of recombination makes fine-mapping possible using a relatively small number of animals. In contrast to recombinant inbred and recombinant congenic strains, each animal in an HS stock is genetically unique.

Intermediate trait and endophenotype: The term 'intermediate trait' is used to describe a heritable quantitative phenotype that is thought to be intermediate in the chain of causality from genes to disease. Such traits are thought be more directly related to etiological factors than dichotomous diagnostic categories. The term 'endophenotype' is sometimes used interchangeably with intermediate trait. But the original meaning of endo-(inside) referred to biochemical traits not measurable in an intact organism, and thus referred only to one specific type of intermediate traits.

Knockin: A type of targeted mutation in which an alteration in gene function other than a loss-of-function allele is produced. Knockin DNA constructs are usually designed to introduce point mutations, which have been proposed to be crucial in gene function or to be responsible for a relevant human trait. Many triplet-repeat disorders, including Huntington disease, have been modeled in knockin mice in which the number of repeats in endogenous mouse genes are manipulated.

Knockout (KO): A type of targeted mutation in which a certain portion of a gene is substituted by a DNA construct to create a loss-of-function allele. The knockout DNA construct is usually designed to eliminate gene function by removing critical domains or introducing frame shift and early termination. In combination with other artificial transgenes, knockout can be tailored tissue-specific and temporally regulated by administering external cues such as tetracycline.

Linkage: The tendency of two loci in close proximity on a chromosome to be separated less frequently than expected by chance (50%).

P50/prepulse inhibition, sensorimotor gating: Sensorimotor gating is a neurophysiological trait that reflects the ability to filter out extraneous stimuli and to process information that comes in rapid succession. In humans, it has been indexed by measuring a small amplitude, positive wave occurring about 50 ms after an auditory stimulus, hence known as P50. When a second sound follows quickly the first one, inhibitory mechanisms are activated and the P50 response is suppressed in most people. This suppression is impaired in many schizophrenic and bipolar patients as well as some of their relatives. It seems to model a diminished capacity to filter out irrelevant stimuli. In mice, sensorimotor gating has been modeled as prepulse inhibition (PPI), which measures attenuation of the startle reflex when a weak stimulus precedes an intense stimulus.

Porsolt forced swim test: In this test, mice are placed in a water tank from which they cannot escape. Initially mice swim vigorously, apparently in a search for an escape route. Later, they occasionally stop swimming and instead float on the surface of the water, thought to be a sign of 'giving up'. The amount of immobile time spent floating is decreased by treatment with antidepressant drugs, supporting that this test is a model of behavioral despair, which might be relevant to depression in humans.

Quantitative trait locus (QTL): Quantitative trait is a phenotype that can vary in a quantitative manner in the population. The variation can be due to combinations of genes and can be affected by environmental factors. Quantitative traits are often controlled by the cumulative (additive) action of alleles at multiple genetic factors, which are called quantitative trait loci. But some QTLs can act only in concert with another QTL due to epistatic genetic interaction.

Recombinant inbred (RI) strain: A special type of inbred mouse strain formed from an initial outcross between two well-characterized parental inbred strains, followed by at least twenty generations of inbreeding. Because each recombinant exists as a strain, correlations between different phenotypes can be studied, and subtle quantitative traits can be mapped because each measure can be repeated in many animals. In addition, most sets of RI strains have been genotyped for markers over the whole genome, so once a phenotype is scored in a set of RI strains, no additional genotyping is necessary to identify the location of genes affecting the trait.

Recombinant congenic (RC) strain: A variation on recombinant inbred mouse strains in which the initial outcross is followed by several generations of backcrossing before inbreeding. RC strains have a higher likelihood that unlinked genes of a multigenic trait would be separated into individual RC strains. Thus, each gene can be mapped and studied independently from the other genes more easily. They have the same advantage of recombinant inbred strains that many identical animals exist.

Single nucleotide polymorphism (SNP): Polymorphism refers to an allele that is frequent (>1%) in the general population. However, rarer genetic variants that do not contribute to the trait of interest are often called rare polymorphisms. Single nucleotide polymorphism refers to any locus where a simple nucleotide change is common in the general population. Although each polymorphism arose once by mutation, the term mutation is nowadays used as disease-associated mutation, and contrasted with (neutral) polymorphism. With quantitative variants affecting subtle traits, this distinction will probably become obsolete in the future.

Social interaction in mice: There are several behavioral paradigms to examine social interaction. The simplest is the observation of whisker trimming and sleeping patterns. When housed together, normal mice usually trim each other's whiskers and sleep huddled together. Figure 3 shows an example of mice deficient in these social interactions. More sophisticated paradigms to examine social interaction are social recognition of males for familiar females, social dominance test between males, and various tests of aggression and fighting in males.

Transgene: A fragment of foreign DNA that has been artificially incorporated into the genome by random integration, usually in multiple copies. A transgene can be designed to be expressed in selective tissues or to be regulated by external cues like tetracycline. Antisense transgenes have been used to reduce or eliminate the normal protein level.

Transmission disequilibrium test: A statistic for association studies. Parents heterozygous for a predisposing allele transmit that allele more frequently to affected offspring than would be expected by chance, i.e., more than 50%. A significant transmission disequilibrium test result means that an allele is both associated and linked with a disorder.

Testing human candidate genes in mouse models for psychiatric disorders

With mouse models of an intermediate trait at hand, mice can be used to confirm genes implicated in human psychiatric disorders, or to determine which of a set of candidate genes might be involved. Most commonly, some evidence for LINKAGE of the human disorder to a chromosomal region is obtained in human populations or families for a psychiatric disorder. Then, candidate genes are evaluated by association studies in humans. In parallel, the same candidate genes can be studied using intermediate traits in mice. This can help rank candidate genes for human studies even when the exact mutations studied are different.

In Table 2 we have summarized recent reports testing specific candidate genes that were implicated in humans, either directly or indirectly by linkage to a homologous region, for their effect on intermediate traits in mouse models. Because no gene involved in a psychiatric illness has been unambiguously identified, the examples in Table 2 should be taken as examples of the approach – none are yet definite models. For example, a very large region on distal

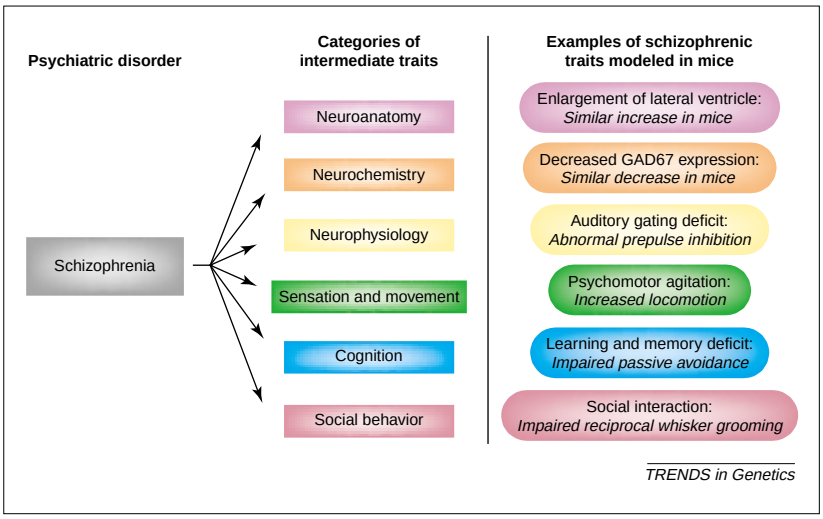

Fig. 1. Categories of traits modeled in mice: example Schizophrenia. A psychiatric disorder is regarded as a black box, impossible to model in mice. However, some of the associated traits, often called intermediate traits, can be modeled in mice. Associated traits from many diverse areas of neuroscience are shown here for the example of schizophrenia. In each box of the right column, an example of a trait associated with schizophrenia is presented in the upper line and the relevant mouse phenotype in the lower line. The specific genes involved in these mouse models are listed in Table 2.

chromosome 7q has been implicated in autism by linkage, but mutations in mice in at least two different candidate genes mapping to that region, *Reln* (Reelin) and *Wnt2*, show deficits in some autism-related traits in the mouse. Sensory gating in humans, measured as P50, is an intermediate trait associated with schizophrenia, and is similar to PPI in mice. Human P50 deficits have been mapped near 15q14, a region containing the gene encoding the α-7 subunit of the acetylcholine receptor (*CHRNA7*) [10]. Inbred mouse strain variations in PPI were found associated with bungarotoxin binding to the receptor, and bungarotoxin binding in turn was mapped in a cross near *Chrna7*, suggesting *CHRNA7* is a good candidate gene for this intermediate trait in humans and mice [11].

Perhaps the best example yet is the implication of *PRODH2* in schizophrenia. Linkage of schizophrenia had been identified on chromosome 22q11–13. In addition, patients with ~3 Mb deletions in this region have velocardiofacial syndrome and often have schizophrenia or another psychiatric diagnosis. *PRODH2* is one of dozens of genes within this 22q11 microdeletion. Gogos *et al.* [12] found that an inbred mouse strain with hyperprolinemia has a nonsense mutation in *Prodh2*, and showed that this strain is normal except for specific defects in sensory gating. In the pursuit of schizophrenia susceptibility factors on 22q11, the same group identified coding variants of *PRODH2* and its neighboring gene, *DGCR6*, associated with schizophrenia using the TRANSMISSION DISEQUILIBRIUM TEST [13]. Although strong linkage disequilibrium in the region did not allow the human association test to discriminate between the effects of the two genes, the fact that *Prodh2* mutations in the mouse affect a trait associated with schizophrenia favors *PRODH2* over *DGCR6*.

Of knockouts, knockins, and ENU mutagenesis

In the example of *Prodh2*, with a microdeletion on one of the two human chromosomes, a mouse heterozygous for a nonsense mutation can be a suitable model for the human condition. In fact, most models available so far are null alleles created using knockout (KO) technology. However, it is unlikely that most human psychiatric disorders are caused by null alleles – quantitative expression variants or amino acid changes that do not inactivate the protein are more likely to be risk factors for common disorders. Sometimes a KO mouse can be a valid model that exaggerates the effect of more subtle point mutations. However, many homozygous KO mice are not viable, precluding the study of more subtle effects of a candidate gene. But, in addition to conventional KOs, there are many ways of manipulating the mouse genome in 'forward' and 'reverse' genetic approaches [14]. Conditional KOs allow investigation of a gene in only a specific tissue or at a specific time, and transgenic manipulations can add more of a gene, or, using antisense RNA, reduce its expression at specified times or tissues [15].

Ideally, specific amino acid changes could be introduced into the mouse as knockin genes, but that is so labor-intensive that it is unrealistic to test hundreds of alleles. Different amino acid changes can have dramatically different, not just milder, effects from null alleles. For example, some channel mutations increase ion flow by changing the kinetics of opening or closing, others change ion selectivity, whereas null mutations eliminate the flux altogether. Therefore, different mutations in the same ion channel can have different phenotypic effects, for example migraine, ataxia or epilepsy, or long QT syndrome and congenital deafness [16].

One approach to find alleles in a specific gene of interest is to search among existing inbred mouse strains, which is how the *Prodh2* mutation was identified [12]. However, the amount of diversity available from existing inbred mouse strains is quite limited, although wild-type mice can contain additional variants. To address this problem, a promising new idea is gene-driven ENU MUTAGENESIS. Rather than screening for a phenotype, we can now screen databases or DNA from progeny of mutated mice or cells for mutations in the gene of interest. Sperm cells of offspring of ENU mutated males can be frozen, and an allelic series of live animals can be obtained after screening their DNA for mutations in a gene of interest [17]. Alternatively, ES cells can be directly mutated with ENU, followed by screening for specific mutations of interest, and mutant animals can then be created directly by injecting the selected ES cell lines [18,19].

These gene-driven approaches should be contrasted with phenotypic mutagenesis screens in which we search for mutant mice that affect the phenotypes of interest, and then identify the gene. In large-scale mutagenesis screens, a trade-off has to be made. To avoid a very large number of false positive mice and to help genetic mapping, only mutations that cause rather

Table 1. Psychiatric disorder-associated intermediate traits that have been successfully modeled in mice[a,b]

Disorders	Deficit	Intermediate traits	
		Humans	**Mice**
Autism	Neuroanatomy	Larger brain volume and weight Decreased Purkinje cells in cerebellum	Reduced cerebellar size
	Motor function	Clumsiness, muscle hypotonia	Motor deficits in hanging wire test
	Neuronal coordination	Frequent seizures	Increase in spontaneous and induced seizures
Schizophrenia Autism	Socialization	Social withdrawal	Impaired social interaction observed by deficits in **reciprocal** whisker grooming or in huddling during sleep
	Psychomotor activation	Psychomotor agitation	Increased locomotor activity in open field
	Stereotypy	Stereotypic behaviors	Stereotypic behaviors; hyperlocomotion in open field or home cage
	Cognition	Learning, memory deficits Cognitive rigidity	Impaired learning and memory in Morris water maze etc Impaired performance in T-maze alternation, or cued or contextual conditioning tasks
Schizophrenia	Gene expression	Decreased GAD67 expression	Decreased GAD67 expression
	Neurochemistry	Reduced prefrontal cortical dopamine release	Reduced prefrontal cortical dopamine release
	Neuroanatomy	Increased ventricle volume	Increased ventricle volume
	Sensorimotor gating	Impaired sensory gating in auditory-evoked potential P50	Decreased tactile or acoustic prepulse inhibition (PPI) and impaired eye blink response
	Working memory	Working memory deficit	Impaired memory in 5-arm maze
Depression	Anxiety	Anxiety	Altered anxiety levels in light/dark box or in elevated plus or zero maze
	Neuroanatomy	Reduced hippocampal volume	Reduced hippocampal volume
	Circadian rhythm	Insomnia or hypersomnia	Abnormalities in rest-activity cycle measured by running wheel or home cage activity patterns
	Energy level, motivation	Fatigability (giving up easily)	Altered behavioral despair measured in Porsolt forced swim test or tail suspension test, or in learned helplessness paradigm
	Cognition	Decreased ability to concentrate and think	Impaired learning in Morris water maze and T-maze
ADHD	Activity levels	Hyperactivity	Increased locomotor activity measured in open field or home cage
OCD	Cleaning compulsions	Excessive Hand washing Trichotillomania	Excessive grooming up to hair loss

[a]Abbreviations: ADHD attention deficit hyperactivity disorder; OCD obsessive compulsive disorder.
[b]For extensive descriptions of mouse assays, see [75].

extreme phenotypes (e.g. three standard deviations from the mean) are selected [20]. By restricting analysis to a small subset of traits (e.g. circadian rhythm), or to a specific chromosomal region, one can afford a lower cut-off, with correspondingly more work to exclude false positives and during genetic crosses, but with an increased chance to identify subtle mutants [21]. Careful adjustment of screening criteria will allow screening for behavioral and intermediate traits for which no mutants exist so far. ENU mutagenesis has resulted in mouse mutants in sensory gating [20] and circadian rhythm [22,23].

Hope and limitations of QTL mapping

In contrast to these approaches, which study the effects of single gene mutations, QUANTITATIVE TRAIT LOCUS (QTL) mapping and cloning uses the variations in existing mouse strains to map loci and eventually clone the underlying genetic variants that affect normal variation in quantitative traits, including the intermediate traits relevant for psychiatric disorder models. QTL mapping has been used extensively in mouse models of drug addiction [5]. QTL mapping has the advantage over the one-genetic-variant-at-a-time approach because EPISTATIC INTERACTION between genes can be detected, for example when one genetic variant's effect is only manifested in the presence of another genetic variant. QTLs are therefore thought to be more realistic models of the complexities in human psychiatric genetics [4]. In addition, many single gene mouse mutants or KO mice show different phenotypes in different genetic backgrounds, often due to a combination of genes. These modifier genes are also of interest and often can be treated as QTLs, indeed some have now been cloned using this method [24].

However, it is very hard to get from a broad QTL to the specific genetic variant, and therefore, only very few complex QTL loci have so far been cloned – none of these are associated with behavioral or psychiatric traits, with the possible exception of sweet taste preference that may be related to alcohol preference [24]. The problem of genetic resolution of QTLs could be overcome by increasing either the number of animals or the number of recombinations detectable in each animal. RECOMBINANT INBRED (RI), RECOMBINANT CONGENIC (RC), and HETEROGENEOUS STOCKS (HS) all increase the number of recombinants per animal by taking advantage of 'historical' recombinants several generations ago. Indeed, MOUSE 'EMOTIONALITY', a psychological trait related to anxiety, has been mapped to 0.8 cM in HS mice [25], and one of the few cloned QTLs, a cancer locus, has been identified with

Table 2. Candidate susceptibility genes for psychiatric disorders that affect relevant intermediate traits in genetic mouse models[a,b,c]

Disorder	Gene	Evidence in human			Evidence in mouse		Refs
		Linkage	Association	Expression	Mouse model	Relevant intermediate phenotypes detected in the given mouse models	
Autism	*EN2*	+	+?		+/– & –/–	Hindbrain and defect; motor learning deficit	[40,44,45]
	GABRB3	+	+		–/–	Frequent seizure; hypersensitivity; hyperactivity; nurturing deficit	[40,46]
	OXT			↓	–/–	Social amnesia, which could be improved by oxytocin injection	[47,48]
Autism/ Schizophrenia	*BDNF*		+?	↑	Tg	Learning impairment; increased seizure severity to kainate	[49,50]
	GRIK2	+	+?	↑/↓	–/–	Deficits in sensorimotor coordination and motivation	[51–53]
	RELN	+	+?	↓	+/–	Age-dependent PPI decrease; neophobia; cerebellar hypoplasia; accumulation of NADPH-d-positive neurons in white matter	[40,54]
	WNT2/ DVL1	+	+?		–/–	PPI deficit; impaired social interaction	[40–42]
Schizophrenia	*CHRNA7*	+	+	↓	DBA	Very poor in PPI; decrease in Chrna7 expression measured by alpha-bungarotoxin binding and associated with a polymorphism in the gene	[10,11]
	DRD3		+?	↓	–/–	Hyperactivity; supersensitivity to cocaine	[55,56]
	GRM5		+?		–/–	Sensorimotor gating deficit; impaired spatial learning and fear conditioning	[57,58]
	NCAM1				–/–	PPI deficit regulated by apomorphine; increase in lateral ventricle size	[59,60]
	NMDAR1	+		↑	kd/kd	Increase in motor activity and stereotypy; social & sexual deficits, which could be improved by antipsychotics	[61–63]
	PRODH2	+	+?	↓	–/–	PPI deficit; neurotransmitter reduction in frontal cortex and hypothalamus	[12,13]
	TNFA	+	+?		Tg	Increased grooming in response to stimuli; impaired acquisition of passive avoidance; impaired learning and memory	[52,64,65]
	ZIC2	+		↑	+/kd	Forebrain anomaly; PPI deficit	[52,66]
Schizophrenia/ OCD	*COMT*	+	+?		+/–	Females show impaired emotional response; males have increased aggression; dopamine increase in frontal cortex	[52,67,68]
ADHD	*DRD4*		+?	↓	–/–	Decreased locomotion but increased reactivity to unconditioned fear; supersensitivity to psychostimulant and ethanol	[69,70]
	DAT1		+		kd/kd	Impaired habituation; hyperactivity reduced by antipsychotics	[71,72]
	SNAP25		+		Cm/+ Tg	Hyperactivity regulated by psychostimulants	[73,74]

[a]Abbreviations: ADHD, attention deficit hyperactivity disorder; OCD, obsessive-compulsive disorder; +, evidence is strong; +?, evidence is weak (tested only once or complicated by negative evidence); ↑/↓ increase/decrease in gene expression; +/–, heterozygote knockout; –/–, homozygote knockout; +/kd, heterozygote knockdown; kd/kd, homozygote knockdown; Tg, transgenic; Cm/+ Tg, heterozygotes of coloboma deletion complemented with SNAP-25 transgene; DBA is a normal inbred strain.

[b]For extensive descriptions of mouse assays, see [75]

[c]Selection criteria: Human evidence for the gene exists from linkage, association, or expression studies. Mouse models for the gene provide at least one intermediate phenotype relevant to the disorder. Models with only neurochemical or neuroanatomical evidence are not listed because of space limit. Many mouse models with altered anxiety exist and meet our criteria for depression, bipolar or anxiety disorders. But they are not listed here because of space limit. Genes are listed with the human locus name.

the help of RC strains [26]. Therefore, an efficient way to increase the number of recombinants for many different studies has recently been proposed: the idea being that ~1000 recombinant inbred strains of eight different strains could be used as a collective resource for fine mapping many different QTLs [27].

Assuming that we might actually be able to identify many mouse QTLs relevant for behavior, how useful will these be? Will they lead us to candidate genes for psychiatric disorders? The answer takes us back to the different phenotypes and traits. Numerous KO mice show effects on anxiety as measured by current methods [28]. It is therefore plausible to assume that the number of candidate genes for anxiety disorders in both humans and mice could potentially be of the order of hundreds of genes. But only a small subset of genes that could affect anxiety in mice is expected to vary significantly between inbred strains. The subset of genes that varies significantly in human populations is therefore likely to be mostly non-overlapping with those fixed in mice – unless we assume evolutionary forces on domesticated inbred mice and human populations were similarly selecting for or against variants in the same genes. If QTL mapping will be used to identify genes thought to be of importance in humans, more specific traits than anxiety and hyperactivity are needed to identify genes of overlapping relevance between humans and mice. Indeed, when a very specific trait such as narcolepsy was considered, mice, dogs and humans all were found to have mutations in the same biological system [29].

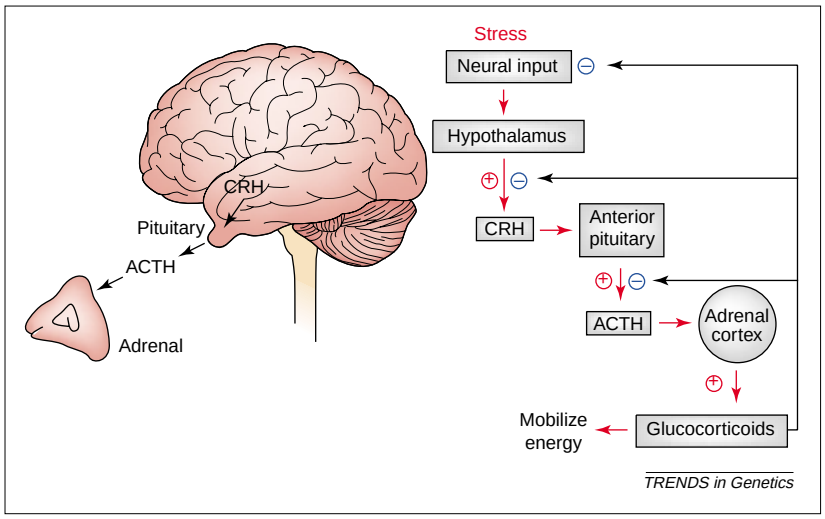

Fig. 2. The hypothalamic-pituitary-adrenal (HPA) axis. The initial changes in the axis in response to stressful stimuli are shown in red. Stress increases corticotropin-releasing hormone (CRH) release from the hypothalamus. CRH binds to CRH receptors on anterior pituitary cells resulting in increased adrenocorticotropic hormone (ACTH) release. ACTH, in turn, is carried via the blood to the adrenal cortex where it stimulates glucocorticoid production and release. Glucocorticoids cause metabolic changes that allow one to respond to the stressor. They also provide negative feedback (shown in blue) to decrease synthesis and release of CRH and ACTH to terminate the stress response and return the system to homeostasis.

Genetic mouse models dissect a system: HPA dysregulation and depression

Once we know a few candidate genes or a good set of intermediate phenotypes in a psychiatric disorder, mice can be extremely valuable to dissect the whole system. The most relevant example for psychiatric disorders is the hypothalamic-pituitary-adrenal (HPA) or stress axis, which has been characterized with several different mouse mutants. Clinically, the association between stress and mood disorders has been known for many years, as stressful events often precipitate depressive episodes in humans. By contrast, the mechanistic ties between the normal physiological response to stress and the pathophysiological state of depression were poorly understood. This changed dramatically with the identification of corticotropin-releasing hormone (CRH) as the key regulator of the endocrine and behavioral responses to stress. In response to stressful stimuli, CRH is released from the hypothalamus and acts as the principal mediator of pituitary adrenocorticotropic hormone (ACTH) secretion [30]. ACTH is carried by the blood to the adrenal gland where it stimulates release of the major stress glucocorticoids, cortisol in humans and corticosterone in rodents (Fig. 2). The glucocorticoids mediate metabolic changes, but are also essential in terminating the stress response, and thus maintain homeostasis by negatively regulating CRH and ACTH synthesis.

Several alterations in the HPA system are observed in major depression including basal hypercortisolemia and inappropriate suppression of the HPA axis by glucocorticoids (reviewed in [31]). These findings suggest that impaired glucocorticoid negative feedback and HPA hyperactivity, most likely caused by hypersecretion of CRH, are hallmarks of depression. Behaviorally, depression is often characterized by increased anxiety, behavioral despair, and altered patterns of activity, sleep and eating – all traits that can be studied in mice. Mouse models with altered levels of corticosteroid feedback or CRH expression and activity can therefore provide key information on the roles of these genes in behavioral traits relevant for depression. CRH-KO mice are viable and grossly indistinguishable from their wild-type littermates, but exhibit a markedly impaired corticosterone response to many types of ACUTE STRESSORS [32]. However, the stress response is not completely absent in the CRH-KO mice, suggesting that other ACTH secretagogues such as vasopressin or catecholamines compensate partially, but not completely, for the absence of CRH in maintaining a stress response. Of significant interest is the stress-induced behavioral responses in the CRH-KO mice. In spite of much literature implicating CRH in anxiety and stress behaviors, CRH-deficient mice exhibit similar measures of anxiety as their wild-type littermates in elevated plus maze and other established anxiety paradigms, and similar decreases in food intake in response to a variety of stressors. CRH receptor 1 (CRH-R1) antagonist studies in these mice suggest that another CRH-like molecule is acting or compensating for the lack of CRH to mediate some of the behavioral responses normally attributed to CRH.

CRH-R1 is expressed in the brain and on anterior pituitary corticotropes where it mediates CRH signaling of ACTH secretion. As predicted, CRH-R1-deficient mice [33,34] exhibit a significantly impaired stress response, similar to the CRH KO mouse. However, unlike the CRH KO mice, the CRH-R1-deficient mice exhibit decreased anxiety-like behaviors. These data suggest that CRH and other CRH-like ligands modulate anxiety-like behavior in the mouse through action on CRH-R1. Similarly, the decreased anxiety-like behavior and reduced stress response in CRH-R1 deficient mice suggest that CRH-R1 antagonists might be effective in treatment of depressive disorders – antagonists are already being tested in small clinical trials [35].

Complementary studies have also examined the effects of altered glucocorticoid receptor (GR) levels in mouse model systems. Together, these models have provided a wealth of information on the roles of specific molecules in HPA function and anxiety-like behaviors, and many compensatory mechanisms in the mouse HPA axis. Although none of these mouse mutants can be viewed as a complete model of human 'depression', they have provided important insights into the regulation and compensatory changes within the stress axis, a physiological system affected by depression, and provided new targets for anti-depressant drugs.

Functional genomic approaches: thinking in pathways

Psychiatric disorders have both environmental and genetic susceptibility factors. The HPA axis can be affected by stress (environment) as well as genetic

manipulations, and both result in similar downstream changes. Thus, not all animal models for a psychiatric disorder have to be the result of genetic manipulation. Assuming that genetic, environmental or pharmacological interventions that result in the same phenotype can merge in a common pathway, microarray techniques can be used to identify these changes and other factors in the same pathway, For example, metamphetamine abuse can induce mania symptoms similar to bipolar disorder. To study bipolar disorder, Niculescu *et al.* [36] therefore treated rats with metamphetamine, and searched for subsequent gene expression changes in several brain regions. Genes that changed in expression were cross-checked against human linkage results to identify candidate genes. This approach identified, among others, *Grk3*, the gene encoding G-protein-coupled receptor kinase 3, which is located in a region of chromosome 22q linked to bipolar disorder [37]. Later, SINGLE NUCELOTIDE POLYMORPHISMS (SNPs) within the promoter of this gene were found associated with the disorder [38].

When several genes are in the same pathway, a mutation in one is likely to have a similar phenotype to a mutation in a downstream gene. KO mice of *Dvl1*, a gene acting in the *Wnt* pathway, did not have any obvious developmental defects but surprisingly exhibited reduced SOCIAL INTERACTION and abnormal sensorimotor gating, intermediate traits of schizophrenia and autism [39] (Fig. 3). In humans, *DVL1* has never been implicated in either illness. Instead, *WNT2*, another key player in the same biological pathway, is localized on chromosome 7q31, one of the regions most consistently implicated in autism [40] which could be involved in a subset of children with autism [41]. Although this is not yet proven, it is just one example of how, once a pathway is identified in mice, all genes in that pathway become candidates for a homologous human disorder.

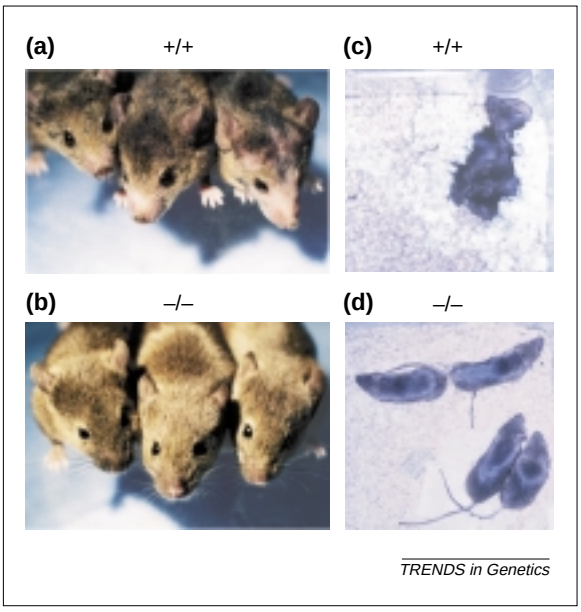

Fig. 3. Abnormal social interaction in *Dishevelled1*-deficient mice. Mice missing the *dishevelled 1* gene (*Dvl1*; –/–) show deficits in social interaction reminiscent of autism or schizophrenia. Full-face views of three +/+ (a) and three –/– (b) cagemates, showing facial whisker patterns. Normal mice groom each others face and whiskers, whereas the hair around the –/– snouts is fully grown, indicating lack of reciprocal grooming. Representative photographs of +/+ (c) and –/– (d) mice, 45 min after the introduction of a nestlet wafer into each cage. Note the fluffy nests built in the +/+ cages and the huddling of mice in these nests, in contrast to the poorly formed nests in –/– cages with random sleeping patterns. (Reproduced with permission from [43]).

Conclusions

Although no mouse will ever completely model all aspects of complex psychiatric disorders such as schizophrenia and autism, many different mouse models, each serving a different purpose, will be useful for the characterization of different aspects of a psychiatric disorder. Intermediate phenotypes associated with each disorder will have an increasingly important role both in human psychiatric genetics and in mouse models. Finding additional, quantitative intermediate traits for both human and mouse studies would be rewarding. One promising area is neuroimaging – both functional and anatomical – which in recent years has resulted in many new psychiatric findings [42]. But very little imaging has been done so far in mice, although this is the kind of quantitative intermediate phenotype we can expect to see more of in the future [43]. Candidate genes for human psychiatric disorders are being tested in mouse models of intermediate phenotypes with some success but very few definite confirmations are so far available. Once a dysfunctional system has been identified, mice will be very valuable in the dissection of complex interactions as well as the identification of additional candidate genes and therapeutic targets, as was discussed with the example of the HPA axis and depression. As our knowledge of pathways affected in psychiatric disorders increases, we expect that such a systems approach towards mouse models for psychiatric disorder will also increase.

Acknowledgements
We thank NIH (NS32130 and DK42730), NARSAD and the Nancy Pritzker Network for Research on Depression for funding. We appreciated the many helpful discussions with many members of the mouse Complex Traits Consortium (CTC, Contact Robert Williams, Univ. of Tennessee for more information), and thank Maja Bucan for helpful comments on the manuscript.

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