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Transgenic Mouse Models and Human Psychiatric Disease

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Summary

Genetic susceptibility to common psychiatric disease arises from the complex interactions between a multitude of genes and an unknown number of relevant environments. However, a common method for investigating gene function involves the creation of a mouse knockout of a candidate gene. Although this approach seems inappropriate to model such complexity, genetic effects on behavior attributable to null mutants in the mouse are in fact subject to the same set of complications, the same gene by environment and epistatic interactions that characterize genetic effects in psychiatric illness. Consideration of the genetic architecture of behavior indicates that even when the molecular lesion is sufficient to inactivate the gene or in other ways alter its function substantially, the effect on the phenotype is typically very mild. Overall, the explanation for the behavior may not be as complex, but it is the product of the same factors. Consequently, it may be possible to take apart the pathway from gene to psychiatric illness.

Key Words: Transgene; psychiatry, QTL; behavior; genetics.

1. INTRODUCTION

The ability to create animal models of human disease by specifically inactivating genes known to play a part in the human condition has transformed the way we investigate the pathophysiology of illness. Obtaining and analyzing a mouse knockout is now a routine part of the functional investigation of genetic disease, and knockout animals are a standard tool in many areas of biology. Nevertheless, using results obtained in a genetically abnormal mouse to solve a problem in human pathology has not always been easy, as is evident in relatively well-understood disorders, such as the inherited anemias *(1,2)*. We can expect interpretation of knockout experiments to be much more problematic when we deal with the behavioral effects of mutations in mice, because we know little about the biology of their behavior. Although there are clearly behavioral domains that are conserved between species, fearfulness for example, others, such as speech disorders, are not. Thus, in some cases, it is not at all clear whether a transgenic mouse will tell us anything about the

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phenotype, a consideration that will not stop those who have identified a gene for dyslexia from making a mouse mutant. Because so much of the genome is conserved between mouse and human species, the expectation is that any knockout will deliver some useful information. Is this really true? In this chapter, the features that complicate the use of transgenic animals for behavioral scientists working with models of psychiatric illness are reviewed.

Presented first are factors that impact the interpretation of any gene knockout experiment: the side effects of the gene knockout technology and the importance of taking into account developmental and tissue-specific effects. However, we are concerned here with factors that more specifically confound investigation of psychiatric disease. These include the compensatory effects of other genes, the difficulties of using a single gene knockout to model a complex phenotype, and the unknown determinants of variation in behavior.

2. EFFECTS CAUSED BY TRANSGENESIS: COMPLICATIONS OF ENGINEERING

The indirect effects from the process used to create the null mutation can be confused with the effects of the induced mutation itself. There are several instances in which disruption of a gene by homologous recombination and the subsequent introduction of exogenous sequence into the genome result in a change in the expression pattern of a neighboring gene or genes. To select for the rare occasions in which homologous recombination produces a knockout, a selectable marker for neomycin resistance is targeted to a crucial portion of the coding region of the gene of interest. This process can itself alter gene expression. Thus, for example, disruption of the *Hoxd-10* homeobox gene (a gene involved in patterning the embryo) leads to altered expression of other *Hox* genes: in embryos, ectopic expression of the *Hox-9* gene was found in the spinal cord of embryos carrying the targeted gene *(3)*. Furthermore in this example, the neomycin gene exhibited a *Hox*-like expression pattern, indicating that its promoter was regulated as if it were a *Hox* promoter *(3)*. Similarly, disruption of two other multigene complexes, the *granzymes B* locus, and the β-*globin* locus control region, also resulted in altered expression of other genes as much as 100-kb distant in the locus downstream insertion *(4)*. Although examples have not been documented for behavioral phenotypes, there is no reason to suppose that they do not exist.

3. EFFECTS CAUSED BY TRANSGENESIS: CONSEQUENCES OF DEVELOPMENTAL CHANGE

Since the introduction of targeted mutagenesis, there has been a concern that the behavioral effects could be a result of the secondary developmental consequences of inactivating the gene, particularly because so many genes implicated in behavior also have a role in development *(5)*. In many cases, the concern has been theoretical, as the accumulated evidence on knockouts influencing tests of spatial memory

have shown: the effects are indeed caused by the mutations and not secondary to other processes. However, there is now one case in which the effect of the mutation has been shown to depend on the time at which it occurs. It has been shown that serotonin (5-hydroxytryptophan [5-HT]) 1a receptors act during development to establish anxiety responses in the adult mouse *(6)*.

The role of serotonin in regulating mood is well established: an increase in the levels of serotonin has an antidepressant effect whereas depleting serotonin is depressogenic. The pharmacological consequences are also well known, as attested by the effectiveness of selective serotonin reuptake inhibitors (such as Prozac) in treating depressive illness. After the discovery in the 1980s that buspirone, a drug that acts at 5-HT1a receptors, is anxiolytic, serotonin has also been implicated in the regulation of anxiety.

From tests of anxiety, mice lacking 5-HT1a receptors seem to be more fearful than wild-type animals. Three groups have made knockouts of the receptor and all reported that the mutants behave in way consistent with a role of the receptors in modulating fear-related behavior *(7–9)*. The knockout mice are less active in tests of novelty (in which the animal is free to explore a novel, potentially threatening environment). All groups concluded that the mutants displayed increased anxiety. However, the data do not necessarily overall support the view that the 5-HT1a receptors are involved in modulating anxiety. Ramboz and colleagues found no significant changes in total serotonin or its metabolites in any region of the central nervous system they examined, suggesting that the behavioral effect was secondary to the genetic lesion *(7)*. Furthermore, the pharmacological data contradict the genetic findings: compounds that block 5-HT1a receptors do not cause anxiety in adult mice.

Rene Hen and colleagues resolved this problem by analyzing a transgenic mouse that they had engineered so that the receptor could be switched off (by feeding the antibiotic, doxycycline, to the animals) *(6)*. They had additionally designed the animal so that the receptor was only expressed in the forebrain, and they were able to show that restoration of receptor function in the forebrain would rescue the anxiety phenotype found in the constitutive knockout (in which the receptor is absent throughout the brain).

Administration of doxycycline to animals aged between 10 and 12 wk eliminated forebrain 5-HT1a receptors in the adult, but had no effect on measures of anxiety. In contrast, administering doxycycline during gestation resulted in animals that had increased levels of anxiety. In other words, the genetic effect depended on the developmental stage; forebrain 5-HT1a receptors are required to modulate anxiety during embryonic and fetal life, but they are not required for that task in adult animals.

This important experiment is still a solitary example, perhaps not surprisingly, given the difficulties of making and using mice with region-specific knockouts under antibiotic control. However, there is no reason to think that the example is unique. Many other behavioral systems may show the same dependence on the timing of the genetic influence on behavior.

4. COMPENSATION

Many of the genes that have attracted the attention of neuroscientists play fundamental roles in a variety of tissues, not just the brain, so it was a considerable surprise to discover that null mutants were not always lethal. In fact, in many cases, the problem has been to find any phenotype at all. One explanation for the relative lack of phenotypic effect is that other genes compensate for the effect of the mutation. It is preferable to call this phenomenon compensation rather than redundancy, because redundancy implies that the gene might have no specific function at all; however, complete absence of a phenotypic effect for a null mutation is unlikely. It is also very difficult to prove that a mutant has no phenotype, because that requires testing the knockout in an almost limitless number of different environments, at different developmental stages, and for different phenotypic effects.

Compensation is not easy to establish, as becomes clear when we examine cases in which the null phenotype in mice is mild, or apparently missing. Four examples of single-gene conditions in which the underlying pathophysiology is relatively well understood, but the behavioral phenotype in the animal model does not replicate the human condition are presented. These examples are gene knockout models for metachromatic leukodystrophy, Lowe syndrome, Tay-Sachs disease, and Lesch-Nyhan syndrome. In each case, the disorder arises from a deficiency in a metabolic pathway and part of the phenotype is mental retardation. However, the behavioral phenotype is not a nonspecific deficit; there is certainly good evidence in Lesch-Nyhan disease that the behavior is so specific that it can be used to make the diagnosis. Thus, it was hoped that mouse models would be able to explain how genetic mutations result in behavioral phenotypes.

4.1. Metachromatic Leukodystrophy

Metachromatic leukodystrophy is a lysosomal sphingolipid storage disorder caused by deficiency of arylsulfatase A (ASA), an enzyme that metabolizes the sphingolipid, cerebroside-3-sulfate (sulfatide), a major lipid component of myelin *(10)*. Deficiency of the enzyme leads to progressive demyelination in the central nervous system. By approx 18 mo, patients present with ataxia and gait disturbance; they go on to develop loss of speech, epileptic seizures, and a spastic quadriplegia. Symptoms are progressive, and children die in a decerebrate state. The behavioral phenotype has some of the features of a psychosis, although, admittedly, the data are limited *(11)*.

ASA-deficient mice, created by transgenesis, have a remarkably mild phenotype compared with humans; they have a normal life span and do not develop widespread demyelination *(12)*. It is not obvious why the animals are so mildly affected as compared with humans. The storage pattern of cerebroside-3-sulfate is comparable in the two species, but gross defects of white matter are not observed in the mouse up to the age of 2 yr. Animals display an astrogliosis and a decreased average axonal diameter and there are abnormalities in Purkinje cells and Bergmann glia of the cerebellum. Demyelination is seen in the acoustic ganglion, resulting in

deafness by 1 yr *(12)*. There is also evidence for a behavioral deficit, but not one that has a clear homolog in humans. Motor coordination and equilibrium is impaired in 12-mo-old ASA-deficient mice, and there are mild impairments in spatial discrimination and fear conditioning (assessed by a Morris water maze and passive avoidance task, respectively), but these impairments are only seen in animals older than 1 yr *(13)*. The decline in neuromotor and cognitive functions in metachromatic leukodystrophy patients is much more severe.

The example of metachromatic leukodystrophy demonstrates that there are unexpected relationships between genotype and phenotype, even in what seem to be relatively well-understood genetic disorders, but the example does not explain how this complexity arises. The next two examples (Lowe syndrome and Tay Sachs disease) indicate the importance of compensatory mechanisms.

4.2. Lowe Syndrome

The oculocerebrorenal syndrome of Lowe (OCRL) is a multisystem disorder affecting the lens (cataracts), and the kidney (resulting in renal Fanconi syndrome). It affects the central nervous system, with some evidence that there is a behavioral phenotype consisting of temper tantrums, stereotypy, stubbornness, obsessions, and unusual preoccupations. Comparison between affected children and controls matched for sex, age, and visual impairment indicated that the phenotype could not be attributed solely to the visual, motor, and intellectual disabilities characteristic of Lowe syndrome, but could represent a specific effect of the mutation *(14)*.

OCRL is an X-linked disorder, and the gene, *Ocrl1*, encodes a phosphatidylinositol 4,5-bisphosphate 5-phosphatase in the Golgi complex *(15)*. Mice deficient in *Ocrl1* do not develop the cataracts, renal Fanconi syndrome, or neurological abnormalities seen in the human disorder *(16)*. One possibility for this surprising result is that *Ocrl1* deficiency is compensated in mice by inositol polyphosphate 5-phosphatase (*Inpp5b*), an autosomal gene that encodes a phosphatidylinositol bisphosphate 5-phosphatase highly homologous to *Ocrl1*. It was possible to test this hypothesis by creating mice deficient in *Inpp5b* and crossing them mice to mice deficient in *Ocrl1*. The double deficiency is lethal, which certainly suggests that the lack of phenotype in *Ocrl1*-deficient mice may be a result of Inpp5b function, but does not help further in providing an adequate mouse model of Lowe syndrome.

4.3. Tay-Sachs Disease

Tay-Sachs and Sandhoff disease are lysosomal storage diseases caused by mutations in, respectively, the *A* and *B* isoforms of β-*hexosaminidase* (*Hex A* and *Hex B*). Disease occurs because GM2 ganglioside accumulates in the central nervous system. The phenotype is variable, but, typically, onset is early and marked by rapidly progressive neurological and behavioral deterioration. The phenotypes of mice with mutations in *Hex A* and *Hex B* do not recapitulate this pattern. As expected, targeted mutagenesis produces *Hex A*-deficient mice that have undetectable levels of β-*hexosaminidase* and accumulate GM2 ganglioside in their central

nervous system in an age-dependent manner and, as in a patient with Tay-Sachs disease, gangliosides accumulate in neurons as cytoplasmic bodies. However, by 3–5 mo of age, the mutant mice show no apparent defects in motor or memory function *(17)*.

Sango and colleagues investigated the relationship between *Hex A* and *Hex B* deficiencies *(18)*. They replicated the finding that *Hex A*-knockout animals are normal; by contrast, the *Hex B*-knockouts displayed progressive deterioration in motor function and gait, to the extent that they were incapacitated by the age of 5 mo. The authors went on to report the phenotype of mice that have both *Hex A* and *Hex B* genes disrupted. Double-knockout mice displayed a total deficiency of all forms of lysosomal β-*hexosaminidase*. Surprisingly, these mice showed the phenotypic, pathological, and biochemical features of the mucopolysaccharidoses, lysosomal storage diseases caused by the accumulation of glycosaminoglycans. The mucopolysaccharidosis phenotype is not seen in the Tay-Sachs or Sandhoff disease model mice, or in the corresponding human patients. They were, therefore, able to argue that the lack of storage of glycosaminoglycans in Tay-Sachs and Sandhoff diseases is a result of functional redundancy in the β-hexosaminidase enzyme system *(18)*.

4.4. Lesch-Nyhan Disease

Lesch-Nyhan disease takes the investigation of compensatory mechanisms one step further. Lesch-Nyhan disease arises from a lack, or very low levels, of hypoxanthine phosophoribosyltransferase (HPRT), an enzyme involved in pathways that resynthesize the components of nucleic acids from their breakdown products. Nucleic acids contain polymers of purine bases that are degraded to urate for excretion or are phosphorylated for further use. The three purine bases involved (adenine, guanine, and hypoxanthine) are served by different enzymes: adenine phosophoribosyltransferase (APRT) works on adenine and HPRT works on guanine and hypoxanthine.

Lesch-Nyhan disease has a remarkably specific behavioral phenotype, characterized by compulsive self-injury, which is present in more than 85% of cases *(19,20)*. The degree and extent of self-injury is remarkably severe: sufferers may require restraint, even teeth extraction, to control the behavior. Typically, injuries occur from biting of lips, fingers, and the inside of the mouth, but affected individuals seek to hurt themselves in other ways as well, even using their own wheelchairs to inflict injuries *(19)*.

Transgenic mice for Lesch-Nyhan disease were created by two groups in 1987 *(21,22)*. The mice seemed normal; there was no evidence of a behavioral deficit *(23)*. One possible explanation for the discrepancy between the mouse and human phenotypes is differential regulation of the nucleotide pool; the relative activities of HPRT and APRT are different in mice, thus, the activity of APRT could be compensating for the deficiency of HPRT.

A simple test of this hypothesis is to inhibit APRT in HPRT knockout mice and see whether a Lesch-Nyhan phenotype arose. Wu and Melton administered 9-

ethyladenine (9-EA), an APRT inhibitor, to HPRT-deficient mice and reported persistent self-injurious behavior in the animals *(24)*. They found that the reduction to 80% of APRT activity relative to the saline-treated control group induced the abnormal behavior. This story seems to provide a good example of how biochemical differences between mouse and human can be overcome and suggests a way forward for other similar discrepancies between mouse and human physiology. Unfortunately, the results are not as straightforward as they seem at first sight.

If the compensation explanation is true, then the same behavioral phenotype observed in the 9-EA-treated HPRT knockouts should arise in a double knockout of HPRT and APRT. Engle and colleagues generated APRT-knockout mice and found that these mice develop kidney stones and renal failure, just as APRT-deficient humans do, confirming that the enzyme has the same role in both species, at least in some tissues. They then crossed the null APRT allele onto an HPRT-deficient mouse background *(25)*. HPRT/APRT double-deficient mice did not exhibit any obvious behavioral abnormalities. They concluded that HPRT/APRT deficiency has nothing to do with self-injurious behavior and is not a good model for Lesch-Nyhan syndrome. A similar conclusion was reached by Edamura and Sasai, who failed to replicate the 9-EA result *(26)*.

In conclusion, although there is evidence in some cases for deficiencies being corrected by known mechanisms, in other cases, the cause of the discrepancy between mouse and human phenotypes is obscure, even when the disturbance occurs in well-characterized biochemical pathways, such as purine degradation and resynthesis. It is evident that we have to further examine why mouse and human phenotypes differ.

5. INTERACTIONS BETWEEN GENES AND ENVIRONMENT

Behavioral geneticists have long stressed the fact that genes and environment work in interaction with each other. Certain phenotypes only emerge when genetically susceptible individuals are placed in a particular environment. Interactions between genes and environment should be easier to examine using knockout mice, and are likely to be important in understanding pathogenesis, but demonstrations of the interactions are still rare.

In human behavioral genetics, a few examples have been reported recently: for instance, Caspi and coworkers reported that the allelic variation at the *serotonin transporter* (*5-HTT*) gene locus moderates the influence of stressful life events on depression. This would explain why some people are more likely than others to develop depression or anxiety after exposure to adversity. It was found that individuals with one particular variant in the promoter of the *5-HTT* gene (known as the short allele) who were exposed to stressful life events were more likely to develop depression than those with an alternative allele (the long allele). It was also reported that childhood adversity predicted adult depression only among individuals carrying a short allele but not among individuals homozygous for the long allele *(27)*.

To date, interactions between genes and environment have been investigated only rarely in the behavioral analysis of mutants. Nevertheless, there is good evidence that such interactions will be found, and the interactions must be taken into account if we are to understand how genetic effects impinge on behavior. Cabib and colleagues demonstrate that a simple environmental change, 12 d of food shortage, can dramatically reverse or abolish differences between inbred strains of mice in behavioral responses to the psychostimulant, amphetamine *(28)*. When food was available *ad libitum,* mice from strains C57BL/6J and DBA/2J exhibited opposite behavioral responses; C57BL/6J mice preferred the place where they previously received amphetamine injections, whereas DBA/2J animals avoided it. Furthermore, amphetamine injection increased locomotor response in C57BL/6J mice more than DBA/2J mice.

Strain differences, however, were altered after several days of food deprivation. DBA/2J mice that experienced food shortage exhibited an amphetamine-induced place preference, and not avoidance, whereas C57BL/6J mice remained unaffected by the food deprivation protocol and continued to show place preference. That is, the strain differences disappeared because of transient food deprivation. Similarly, locomotor responses were also modified by food shortage, and the changes were strain dependent. Whereas C57BL/6J mice were again unaffected by previous food deprivation, DBA/2J mice became highly responsive to amphetamine injection and showed a robust elevation of activity.

This is one example that demonstrates how important a more sophisticated investigation of behavior in mutants must become to dissect the pathway from genetic lesion to behavior. There are likely to be other complex interactions between the induced mutation and the environment that have yet to be discovered. However, this is still only part of the story. It is also necessary to take into account the genetic background of the animal carrying the induced mutation. The effect that other genetic variants have on the behavioral phenotype of a transgenic mouse will be discussed next.

6. BACKGROUND GENES

The importance of genetic background has been appreciated for some time. Threadgill and colleagues' report in 1995 showed that, on a 129/Sv background, homozygous mutants for epidermal growth factor receptor died at mid-gestation because of placental defects, whereas, on a different genetic background (CD-1), the mutants lived up to 3 wk and showed abnormalities in the skin, kidney, brain, liver, and gastrointestinal tract *(29)*. Simply put, genetic variants at loci distinct from the null allele modify the phenotype, potentially vitiating a comparison between knockout animals and controls, as pointed out by Gerlai *(30,31)*.

Targeted mutations in mice are commonly made in embryonic stem (ES) cells derived from the 129 mouse strain and introduced into a blastocyst to generate chimeric embryos. Offspring are mated to wild-type (nonmutated) mice in the hope of obtaining germline transmission of the mutation. Often, mating is carried out

with a different inbred strain, normally C57BL/6; thus, the offspring inherit one chromosome homolog from C57BL/6 and the other from the 129 strain. Consequently, the offspring are not only heterozygous for the null mutant allele but are also heterozygous at all loci that are allelic between strains 129 and C57BL/6. There are also differences between substrains of 129 mice, therefore, the effects will vary depending on the origin of the ES cells *(32,33)*.

The strain effects on behavior are important because the 129 mouse has an unusual behavioral profile. Moreover, strain 129 mice suffer from dysgenesis of the corpus callosum and possess a number of other neuroanatomical abnormalities *(34)*. The animals are impaired in spatial learning tasks, which are frequently assessed in behavioral profiling of mutants, and the strain has a relatively high emotional reactivity (for example, they show relatively increased conditioned freezing, and low levels of exploratory activity in tests of novelty) *(35–38)*.

One specific example will serve to demonstrate the importance of appreciating the behavior of the 129 strain. Dockstader and van der Kooy examined the rewarding effects of psychoactive drugs in the 129/SvJ and C57BL/6 mouse strains using the place-preference paradigm mentioned in Section 5. *(39)*. After completing four conditioning trials, C57BL/6 mice preferred the chamber in which they previously received morphine injection to the nonrewarded chamber. Using the same paradigm, morphine was found to be rewarding for the 129/SvJ mouse strain, but only when the animals were under the influence of morphine during testing. Did this represent a difference in drug response, motivation, or learning between the strains? Further experiments revealed that the inability of 129/SvJ mice to exhibit morphine-rewarded place preference could be fully reversed by a pretest injection of anxiolytic agents (diazepam and pentobarbital), implying that the strain's behavioral phenotype was caused by anxiety.

Elevated anxiety in the 129 can certainly confound interpretation of gene-targeting experiments, as Holmes and colleagues report. They investigated anxiety-like behavior in mice with a null allele of the *5-HTT* gene. On a C57BL/6 background, null mutants exhibited increased anxiety-like behavior and reduced exploratory locomotion *(40)*. Comparison of *5-HTT* mutants on a C57BL/6 with 129S6 congenic background revealed that the mutation did not manifest as an alteration in fear-like behavior on the 129S6 background. The authors concluded that high baseline anxiety-like behavior in the 129S6 strain could indeed have precluded detection of the anxiety-like effects of the *5-HTT*-null mutation *(41)*. It should also be noted that there are differences in anxiety-like behavior between substrains of 129 mice *(33)*.

One accepted way of dealing with the problem of genetic background is to compare the mutant with its wild-type littermates, because, taken together, the same loci will be segregating in the mutants as in the littermates. Although, individually, no two animals are identical, by including enough animals in the two groups (those with and without the mutation) the effect of the mutation can be separated out from other, independently segregating loci. If the effect of the mutation is large (for instance large enough to account for 50% or more of the phenotypic variation),

then a dozen animals in each group will be enough (assuming the background genetic effects are of the usual magnitude of a few percent variance attributable to each locus). However, when the mutation has a small effect, much larger groups will be needed.

An alternative way of dealing with background genetic effects is to backcross the mutant to one parental inbred strain for sufficient generations to purge the genome of all variants. Then, comparisons can be carried out between the knockout and the relevant pure inbred. This approach works well when the parental strain is the same as the strain from which the ES cells are derived (the 129 strain), but becomes complicated in the more usual situation when the knockout is crossed onto a different strain (C57BL/6). In this case, it is almost impossible to remove regions of the genome physically close to the mutation. To do so requires obtaining recombinants that occur precisely at either side of the site of the knockout, which is extremely unlikely to happen. As Bolivar and Flaherty point out, the knockout is actually a form of congenic, an animal that contains one small chromosomal segment from one strain, and the rest of the genome from another *(42)*. Obviously, if the introgressed segment contains no variants that influence the phenotype, the effect is irrelevant. Unfortunately, most behaviors in mice are influenced by many genes *(43)*, therefore, the probability that the segment contains an allele with an effect is not negligible. It may be unlikely, but the problem cannot be ignored.

There are now numerous examples in which behavior in knockouts depends on the strain background *(44–46)*. For instance, neuronal nitric oxide synthase knockout males were first tested for aggression in a mixed 129/SV and C57BL/6J background, and found to be highly aggressive as compared with wild-type littermates *(47)*. However, after five backcrosses into C57BL/6J, the neuronal nitric oxide synthase knockout male offspring were no more aggressive than wild-type littermates *(48)*. Similar findings have been reported across a range of behaviors, including spatial learning *(49)*. Differences observed between mutant and control mice could be caused by the genetic differences between the inbred strains used in the generation of null mutant animals and not by the null mutation itself. For instance, Kelly and colleagues analyzed a mouse with a knockout of the dopamine D2 receptor and found that wild-type strain 129 mice with unaltered functional D2 receptors had the same locomotor deficits as those attributable to the mutation *(45)*. Analysis of mutant congenic strains (backcrossed to either B6 or 129 parental strains) showed a significant interaction between background genes and the targeted mutation, the former exhibiting a greater effect on the behavioral phenotype.

Effects of 129 alleles have also been documented: for instance Errijgers and Kooy review evidence that variation in test results of the *fragile X* knockout mouse depends on the residual effect of 129 alleles in the C57BL/6 background *(50)*. Paradee and colleagues present evidence of the 129 genetic effects on tests of visuo–spatial orientation *(51)*.

Finally, in a few cases, investigators have crossed a mutant onto different strains to assess the effect of background genes. For example, the estrogen receptor (ER)-

α-knockout mouse was examined on C57BL/6J, DBA/2J, BALB/c, and A/J strains, and dramatic effects on male sexual behavior were observed. ER-α-knockout males in the DBA/2J and BALB/c backcrosses displayed more intromissions compared with males in the C57BL/6J and A/J mixed background. Many fewer ER-α-knockout females than males displayed masculine sexual behavior in any of the three hybrid crosses *(52)*.

Background effects are important in any phenotype: the example given in Section 6 of the epidermal growth factor receptor makes this point. However, there is evidence that the effects are of particular concern to behavioral genetics. Detecting the effect of the null allele using littermate controls depends on the effect size of the mutation; large effects are less likely to be obscured by background effects, which usually consist of many small-effect loci *(43)*. As a rule, null alleles have a small effect on behavioral phenotypes. It is possible to work out the effect size from the means and standard deviations reported for each genotype (wild-type, heterozygote, and homozygote mutant), data which are generally reported with the behavioral analyses. For example, using the published data on the effect attributable to the corticotropin-releasing hormone receptor-2 knockouts *(53)*, the knockout allele accounts for approx 10% of the total phenotypic variation, a figure consistent with the size of naturally occurring genetic effects that contribute to individual differences in behavior *(43)*.

Of course, it could be argued that the lack of large effects merely reflects the relatively small number of available knockouts. If more genes were inactivated, then we might find examples of mutations that have a large effect on behavior. However, this argument can be countered by the relative failure to detect segregating behavioral mutations in the mouse mutagenesis projects; that is, the analysis of single-gene mutations systematically produced in mice through the administration of a highly mutagenic compound *N*-ethyl-*N*-nitrosourea. *N*-ethyl-*N*-nitrosourea introduces single basepair mutations randomly at a rate sufficiently high to make it realistic to screen for the effects of mutations *(54,55)*.

Results from the mouse mutagenesis projects suggest that the yield of behavioral mutants is less than expected. Behavioral assays were included in four mutagenesis screens, covering learning and memory, motor activity, fear (or anxiety)-related behaviors, and a test of sensorimotor gating (prepulse inhibition, a model of one aspect of schizophrenia). Three screens have now discarded these behavioral assays because of the low yield of heritable mutants. More than 10,000 mice were screened at the Medical Research Council mammalian genetics unit in Harwell for prepulse inhibition deficits, however, no mutants were found. Similarly low yields are reported from other screens (http://www.gsf.de/ieg/groups/enu/behaviour.html, http://www.neuromice.org/, and http://www.mgu.har.mrc.ac.uk/).

How many functional mutations would we expect the mutagenesis projects to have detected? Sequence analysis of 370 kb of DNA of mutagenized mice detected six sequence changes, suggesting that a mutation will be found approximately every 60 kb *(56)*. Using a per locus functional mutation rate of 1.08×10^{-3}, the probabil-

ity of obtaining at least one mutant is 0.66 if 1000 animals are screened, 0.86 for 2000 animals, and 0.97 for 3000 animals. Because the largest screen has processed 10,000 mice a year (http://www.neuromice.org/), the lack of inherited mutations with an effect on behavior cannot be explained by inadequate numbers of animals. The likely explanation is the relatively small size of the genetic effects.

If, as these observations suggest, genetic effects on behavior are small, it raises the possibility that we rarely, if ever, observe an effect that is independent of background genes. It may be the case that the outcomes we see in knockouts are more complex than we imagine, and that, in fact, knockout mice model the genetic susceptibility to psychiatric disease more closely than has been suspected. We are misled by the analysis of null alleles into thinking that the abnormal phenotype is the product of a single mutation; it may be nearer the truth to say that the effect is polygenic (caused by the induced mutation in combination with effects from many other loci). As discussed in Section 7, this model approximates much better the genetic susceptibility to psychiatric disease than a single gene knockout. Although it is true that detecting and controlling for background genetic effects is an important issue in the behavioral analysis of knockout animals, it may also be true that we need to look more closely at the interaction between modifier loci and the knockout if we are to understand fully how genetic lesions produce disease, a point made by Errijgers and Kooy in their review of the difficulties of modeling the fragile X syndrome *(50)*.

7. COMPLEX GENETICS

Apart from the difficulties of determining which parts of a psychiatric phenotype can be modeled in mice, we need to decide whether the genetic models in knockout mice are at all comparable to those in human psychiatric disease. Genetic susceptibility to psychiatric disorder arises from the conjoint effect of many loci, each contributing only a relatively small amount to the total genetic liability, and it is likely that part of that liability is caused by interaction between genetic loci (epistasis).

The full complexity of genetic architecture of human behavioral variation is still not clear, but there is very little evidence that major gene effects influence psychiatric disease. Despite considerable effort to find families, there are no convincing reports that psychiatric disorders are caused by mutations in a single gene. There are a few instances in which psychiatric illness arises in the context of a genetically determined syndrome. For example, patients with a deletion on chromosome 22q giving rise to velocardiofacial syndrome also have psychotic symptoms *(57)*, but no one has published a pedigree with a segregating recessive or dominant mutation that gives rise to the common psychiatric conditions of anxiety, affective, or psychotic disorders.

The number of genes involved in a common psychiatric illness is unknown; using linkage data collected on a large set of affected sibling pairs, Neil Risch and colleagues put a lower boundary on the number of susceptibility loci for autism at

approximately a dozen loci *(58)*, but the results for this, and other data sets are also compatible with the presence of hundreds of loci. Additionally, the mode of action of each locus is unknown; it is suspected that there may be considerable, but so far undetected, amounts of interaction between loci in many complex phenotypes (not just psychiatric illness, in fact). Again, autism provides an example, in which there is a concordance between monozygotic twins (those that are genetically identical) of approx 90%, whereas the concordance in dizygotic twins (who share half their genes) is only approx 10% *(59)*. Under a simple additive model, in which the phenotype is the outcome of the independent action of each locus, we would expect the concordance in dizygotic twins to be approximately half that found in monozygotic twins. The discrepancy can be accounted for by gene interaction (although there are other explanations).

In addition to the number of loci involved and their mode of action, the complex relationship between genetic susceptibility and phenotype in psychiatric illness has to be taken into consideration when assessing the suitability of a genetic model. In some cases, susceptibility seems to have a quantitative nature; thus, for example, rates of depression are higher in first-degree relatives of a patient with recurrent major depression than in unrelated controls. A pattern of this sort is consistent with additive genetic action, in which a number of loci contribute to increase susceptibility to the disorder. However, psychiatric genetics also has examples of patterns that are not so easily explained. For example, studies of psychosis show that there is phenotypic spectrum in first-degree relatives.

Seymour Kety introduced the term *schizophrenia spectrum* to refer to all disorders that are "to some extent genetically transmitted" with schizophrenia *(60)*, and, since then, there have been efforts to develop operational definitions of the schizophrenia spectrum, resulting in the appearance of criteria for a number of personality disorders (schizotypal, schizoid, avoidant, and paranoid personality disorders). Subsequent work has set out to determine whether there is indeed a genetic relationship between schizophrenia and the spectrum disorders. Three studies have examined the risk of developing schizophrenia in offspring reared by biological parents with schizophrenia *(61–64)*. All report an increase in the genetic liability for schizophrenia-related illness. Similar observations arise from studies of first-degree relatives *(65)*. Together, these reports indicate that the genetic liability is not restricted to narrowly defined, typical schizophrenia but includes schizotypal and schizoid personality disorders and nonschizophrenic nonaffective psychoses.

Knockout animals obviously cannot capture the multilocus and "spectrum" pattern of susceptibility that characterizes psychiatric genetics, because, in a knockout, the genetic liability is caused by a single gene. However, as discussed in Section 6, the phenotypic consequences of the mutation do not arise simply; the effects are frequently small and often depend on background genetic variants for the appearance of the phenotype. This aspect of the behavioral outcome of transgenesis has received little attention, but it may provide new avenues of investigating the pathogenesis of psychiatric illness, allowing us to explore the issues of

complexity in a new way. It may also explain the confusion and contradictory results in current findings.

Some years ago, Sanes and Lichtman, observed that approx 100 genes had been implicated in a cellular phenomenon called long-term potentiation thought to underlie memory processes in the hippocampus *(66)*. How, they asked, could so many apparently unrelated molecules be said to explain long-term potentiation? Clearly, this is a challenge to the causal model adopted from biochemical genetics, in which genes can be arranged in simple linear pathways.

An alternative model is to look at the interactions of the gene products, the proteins, as has been achieved in yeast genetics *(67–71)*. Analysis of the patterns of interactions showed that it was possible to determine structure in the network, leading Barabasi to propose that protein interactions involved in metabolism had the properties of a scale-free network *(72)*. In a scale-free network, the components are organized in the same way as the Internet, or as airlines that connect different destinations via a series of hubs. Because of this feature, scale-free networks are robust and error tolerant *(73)*.

Grant and colleagues have asked whether similar networks apply for proteins involved in behavior, using an excitatory neurotransmitter receptor (the *N*-methyl-D-aspartate receptor) as a model system for investigating the nature of protein (and gene) interactions *(74–76)*. They found that the interactions did indeed fit the pattern of a scale-free network; furthermore, when they mapped the effects of knockouts onto the network they found that mutations which affected a hub had, as expected, much more profound effects than mutations that affected the spokes of the network. Rather than manifesting with a large effect on behavior, knockouts that involved hub genes were likely to be lethal. These initial forays into a network analysis of behavior give some idea of how further analyses could proceed, providing us with a much richer, more sophisticated picture of the relationship between mutation and behavior.

8. CONCLUSION

Transgenic mouse models have undoubtedly made immense contributions to our understanding of behavioral disorders and to neurobiology in general *(77)*. Their value is seen as a tool for the investigation of gene function; by inactivating a gene we can observe the consequences and infer what that gene does in the intact animal. At a time when we are beginning to identify the molecular components of disorders such as schizophrenia, for which we have few clues about the biology, the availability of transgenic animals is a great advantage, an indispensable resource for working out what genes do *(78)*.

The difficulties of engineering mice so that they have mutations that affect only the gene of interest are well-known and have been discussed in this chapter. They include the direct effects of the induced mutation, such as its ability to influence expression of neighboring genes in addition to the target, and the importance of developmental and tissue-specific effects. The complications that influence inter-

pretation of behavioral experiments are also well appreciated. Because we know so little about the biological origins of behavior, we rely substantially on behavioral measurements to assess the nature of the mutant phenotype. However, when we use an outcome measure that is so distant from the molecular lesion, it becomes extremely difficult to observe the pathway that leads from mutation to phenotype. A host of intermediate influences, ranging from other genes, to cellular systems, and, finally, to environmental effects, combine to obscure the relationship between gene and behavior.

As I have attempted to explain in this chapter, a strictly reductionist analysis of knockouts is not necessarily the best way to proceed when tackling models of psychiatric illness. No doubt, when there are specific hypotheses to test about gene function in a well-understood system, this model is appropriate; although, as we have seen in the discussion on compensation, even in these circumstances, the phenotype of a knockout experiment can confound expectations. It is sobering to realize that we still do not understand why the phenotype of one of the first genes ever to be knocked out in mice, HPRT, is not the same as its human equivalent (Lesch-Nyhan disease), even though we know much about the biochemical pathway that has been disrupted.

I have suggested that there are alternative ways of using knockout animals. A consideration of how genetic effects operate in psychiatric disease leads to the now undeniable conclusion that genes of large effect are rare and probably nonexistent for many common psychiatric conditions. Instead, we see that genetic susceptibility to common psychiatric disease arises from the complex interactions between a multitude of genes and an unknown number of relevant environments. On the face of it, using the single-gene model that we have available in the mouse knockout seems completely inappropriate to model such complexity. However, genetic effects on behavior attributable to null mutants in the mouse are in fact subject to the same set of complications, the same gene by environment, and epistatic interactions. Consideration of the genetic architecture of behavior indicates that even when the molecular lesion is sufficient to inactivate the gene, or in other ways alter its function substantially, the effect on the phenotype is typically very mild. Overall, the explanation for the behavior may not be as complex, but it is the product of the same factors. Consequently, it may be possible to take apart the pathway from gene to psychiatric illness. Using networks of interactions to understand how genes and then proteins work, it will be possible to begin to explain cellular processes, and eventually to explain how cellular processes give rise to behavioral phenotypes.

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