Single-gene influences on brain and behavior

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Abstract:
As traditional behavioral genetics analysis merges with neurogenetics, the field of neurobehavioral genetics, focusing on single-gene effects, comes into being. New biotechnology has greatly accelerated gene discovery and the study of gene function in relation to brain and behavior. More than 7,000 genes in mice and 10,000 in humans have now been documented, and extensive information about the genetics of several species is readily available on the World Wide Web. Based on knowledge of the DNA sequence of a gene, a targeted mutation with the capacity to disable it can be created. These knockouts--also called null mutants-- are employed in the study of a wide range of phenotypes, including learning and memory, appetite and obesity, and circadian rhythms. The era of examining single-gene effects from a reductionistic perspective is waning, and research with interacting arrays of genes in various environmental contexts is demonstrating a need for systems-oriented theory.
Key Words: Human Genome Project, quantitative trait locus, reductionism, World Wide Web, targeted mutation

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INTRODUCTION

Unlike previous behavioral genetics reviews in this series, which divided the field by species (human and nonhuman) (Wimer & Wimer 1985, Rose 1995), this review divides the field according to single-gene and biometrical methodologies. It focuses on remarkable progress and prospects in the discovery and understanding of specific gene effects in several species, including humans. Many neurological mutations exhibiting large effects have had their DNA sequences decoded and their protein products identified, and much has been learned about how gene expression is regulated by the environment. Behavioral genetics researchers have advanced to a new stage, and have now begun to examine interacting pairs of genes and to identify viable genetic variants that exert more subtle effects on behavior. As the field of neurobehavioral genetics emerges, genetic tools are becoming central to research in physiological psychology.

Typically, the biometrical or quantitative genetic approach is applied when many unknown genes, each with presumably small effect, are believed to be involved. Instead of identifying specific genes, this methodology seeks to partition variance among several components attributable to genetic and environmental variation. There has been a tension between the two approaches since the early days of genetics, as reflected in Johannsen's (1911:138) opinion of the correlational methods employed by Francis Galton and Karl Pearson: "They have nothing at all to do with genetics--or general biology! Their premises are inadequate for insight into the nature of heredity." This tension continues. Although the mathematical models of biometrics have undoubtedly become more sophisticated and are being applied to both nervous system and behavioral analysis (Rijsdijk & Boomsma 1997), fundamental disagreements abound concerning the basic formulation and assumptions of the quantitative models (Devlin et al 1997, Schonemann 1997, Wahlsten 1990, 1994), and many practitioners of quantitative genetics are being drawn to the study of single-gene effects (Boomsma et al 1997, McClearn et al 1991). In the view of Plomin and associates (1994), "additional quantitative genetic studies are no longer needed to document the importance of genetic influence" (i.e. heritability) on intelligence, and researchers should instead attempt to identify specific genes.

GENE DISCOVERY

The ultimate goal of behavioral and neural genetics is a comprehensive understanding of the identities, functions, and multifarious relations of genes relevant to the behavior of organisms. In this regard, it is important to know how many distinct genes a species possesses, how many of these have already been identified, and how many are likely to be important for behavior.

The Compleat Genome

The Human Genome Project seeks to determine the entire sequence of the nucleotide bases (A, C, G, T, or adenine, cytosine, guanine, thymine) in the DNA of the chromosomes of several species. The overall size of the genome in terms of millions of bases (Mb) is listed for several species in Table 1. Once the entire DNA sequence is known, molecular biologists can identify every unique gene by noting the telltale signatures of base sequences that indicate where to start and stop the transcription of DNA into messenger RNA (mRNA). Each mRNA molecule is translated into a protein molecule. If one knows the DNA sequence of a gene, the structure of its corresponding protein can be readily deduced from the genetic code. This has been accomplished in several unicellular organisms, including a yeast with 6297 genes.

Table 1 ***Size of the genome in relation to the number of genes, proteins, and neurons in several species that are intensively studied in behavior genetics(a)***

Species	Genome (Mb)	Genes	Known proteins	Nerve cells
Yeast (Saccharomyces cerevisiae)	13.50	6,297	6,297	1 cell
Nematode worm (Caenorhabditis elegans)	100	14,000	11,274	302
Fruit fly (Drosophila melanogaster)	165	12,000	1,566	250,000
House mouse (Mus domesticus)	3,300	70,000	7,161	40 million
Human being (Homo sapiens)	3,300	70,000	11,060	85 billion

Species	Web sites
Yeast (Saccharomyces cerevisiae)	genome-www.stanford.edu
Nematode worm (Caenorhabditis elegans)	elegans.swmed.edu
Fruit fly (Drosophila melanogaster)	flybase.bio.indiana.edu
House mouse (Mus domesticus)	www.informatics.jax.org; biomednet.com
Human being (Homo sapiens)	bioinfo.weizmann.ac.il/cards; gdbwww.gdb.org; www3.ncbi.nlm.nih.gov/Omim

(a) Sources: Miklos & Rubin (1996), Henikoff et al (1997), Gottlieb (1998).

The task of sequencing is immensely more tedious in vertebrates because the segments of DNA (exons) that are transcribed into mRNA and translated into protein are interspersed by numerous and large segments (introns) that do not code for protein. It is estimated that in humans and mice the informative exons comprise a paltry 2% of the total DNA; a fabulously expensive effort to date has completed the sequencing for only 2% of the human and 0.2% of the mouse genomes (Rowen et al 1997). Once the numbers of genes in DNA that has already been sequenced are known, the total number can be estimated (Table 1). The process of gene identification in

vertebrates can be greatly accelerated by studying the mRNA expressed in a variety of tissues from different age groups. This mRNA can be reverse-transcribed into complementary DNA (cDNA), which consists entirely of exons that can be analyzed to yield expressed sequence tags (ESTs). This has been accomplished on a large scale for the human genome, and ESTs exist for one or more exons of some 40,000 of the expected total of 70,000 human genes (Rowen et al 1997). Progress toward a relatively complete accounting of expressed genes can be assessed by the number of protein structures known in a species (Table 1).

Sequencing the genome of convenient, nonhuman species has major benefits for gene discovery in humans because many genes and proteins are homologous owing to descent from a common but remote ancestor. For example, at least 1914 of the 6297 proteins of the yeast S. cerevisiae have homologs in mammals (Botstein et al 1997), and homology is substantially greater in more closely related species such as mice and humans (see www.informatics.jax.org/reports.html for an extensive list of homologies).

Targeted Mutations

Of the tens of thousands of genes in a mammal, how many might be relevant for understanding nervous system development and behavior? This question can be approached directly. Once the DNA sequence of an exon of a gene is known, a custom DNA probe can be constructed and then inserted into that specific gene (Joyner 1993). This procedure creates a targeted mutation that usually prevents synthesis of the corresponding protein (called a knockout, or null mutation), but it is also possible to change only one specific amino acid in a protein (Giese et al 1998). The mouse is the preferred subject for this technique, and the 129 inbred strain is commonly the source of cells that are genetically altered. Because one common substrain (129/SvJ) has been genetically contaminated (Simpson et al 1997, Threadgill et al 1997) and the 129 strain, like all inbred strains, has a number of neural and behavioral abnormalities, interpretation of results is sometimes clouded (Crawley et al 1997, Gerlai 1996, Wahlsten & Sparks 1995, Wolfer et al 1997). Nevertheless, the knockout technique is invaluable and can be refined to address earlier shortcomings. Hundreds of kinds of mice have been created that lack a specific protein (such as the estrogen receptor from the Estra gene), and numerous mouse models of human genetic diseases have been created by altering the relevant gene (e.g. the Fmr1 knockout model of Fragile X mental retardation). The null mutation is a relatively blunt instrument, but in many instances researchers have been surprised to obtain viable animals that experienced only minor damaging effects or showed no perceptible effects at all. For example, mice with a disabled dopamine [Beta]-hydroxylase gene (Dbh) are unable to synthesize norepinephrine and have motor difficulties, but are otherwise able to learn reasonably well (Thomas & Palmiter 1997). Because the use of small sample sizes is common in work with knockout mice, most such experiments lack statistical power to detect small or moderate effects and make it risky to proclaim the genesis of a completely normal mouse. Furthermore, researchers usually focus on one phenotype and target genes of particular interest, implying that the extant sample of mutations is not at all representative of the mouse genome. A functional scan of the entire genome by knocking out one gene at a time is now feasible for yeast; in the near future the scan may also be applicable to nematode worms, but not to more complex animals. Another approach is to create random mutations (many of which will occur in unknown genes) and record how many of these then impair development of an organ. Although a precise number cannot be ascertained at present, available data suggest that thousands of genes--perhaps as many as 70% of all genes--are required for the normal development of a complex organ such as the eye (Miklos & Rubin 1996).

Linkage and Chromosome Mapping

DNA-based technology can reveal all genes, whether or not they have alternate forms (alleles) that create protein polymorphism, or individual differences in behavior in a population. Consequently, much of this genetic information is of less interest to psychologists for whom the relatively few genes pertinent to behavioral disorders provide more relevant information. The classical approach to genetics begins with a noteworthy difference in phenotype and then asks whether inheritance follows Mendelian rules and whether the hypothetical gene is linked to a marker at a known location on a chromosome. The search for linkage has been greatly facilitated by the discovery of thousands of phenotypically neutral and highly polymorphic DNA markers scattered widely across the genome of mammals (Dietrich et al 1995). If a mutation in an unknown

gene with major effects on brain or behavior occurs, it is now possible to detect it quickly and locate it accurately on a chromosome map.

A good example is provided in mice by the barrelless (brl) mutation that obliterates the normal barrel-shaped pattern of neuron assemblies in somatosensory cortex. The first description of the phenotype was published recently (Welker et al 1996), and fewer than 2 years later it was mapped to a narrow zone on chromosome 11 (Abdel-Majid et al 1998) that was already known to contain six other genes. These six became plausible candidates for brl, and the search quickly narrowed to focus on the gene Adcy1, which codes for the enzyme adenylyl cyclase type I, an important part of an intracellular signalling pathway involving cyclic AMP (cAMP) in neurons. As it turned out, that enzyme had reduced activity in the mutant mice, and an unrelated knockout strain that lacked a functional Adcy1 gene was found to lack the brain barrels. The mutant mice also suffered memory deficits. The causes of the barrel structures were also clarified. This distinct pattern is impressed on the cerebral cortex by neural input from the vibrissae in the animal's snout, and alteration of the cAMP pathway by a mutation prevents the anatomical imprint of sensory experience. Now that the gene is better understood, it is properly referred to as the adenylyl cyclase type I gene, and the mutation becomes the loss of function allelle [Adcy1.sup.brl].

Genetic mapping with neutral markers also works well in human subjects and has recently been used to detect genes pertinent to many rare neurological disorders. Most cases of unequivocally successful mapping of disease genes have involved dichotomous phenotypes that differ distinctly between normal and abnormal individuals, and where the mutation has a large effect. Tremendous efforts have been made to detect linkage with hypothetical genes pertaining to some of the more common psychiatric disorders that fall into rather arbitrary diagnostic categories, such as manic depression and schizophrenia. Several published claims of linkage have proven to be false positives, and the most recent evidence for linkage of schizophrenia with markers on chromosomes 6 and 8 remains only weakly suggestive (Kidd 1997, Moldin 1997, Moldin & Gottesman 1997). Given the many studies done on this topic, it is reasonable to conclude that no single gene contributes in a major way to the etiology of schizophrenia. Any genetic influence most likely involves the "nonlinear interaction of multiple genetic and environmental factors" (Cloninger 1997), and these effects will be very difficult to identify with conventional linkage analysis that assumes all effects to be independent (Kidd 1997).

Quantitative Trait Loci

Most behavioral variation is continuous and most genetic effects are probably not very large in the normal range of variation. A moderate effect size of a quantitative trait locus (QTL) can be detected by its linkage with neutral DNA markers (Belknap et al 1997, Lander & Schork 1994, McClearn et al 1991). The results are most readily interpreted when the experiment begins with two inbred strains because there can only be two alleles, and the marker alleles will be known in both strains. In an [F.sub.2] hybrid cross, genotype frequencies will have Mendelian ratios at the marker. The closer the marker locus is to the hypothesized gene, the lower will be their recombination probability. Thus, if the QTL has an appreciable effect on behavior, there should be a statistically significant difference in mean behavioral test scores of individuals with different marker genotypes. By examining several markers on the same chromosome and using the MAPMAKER computer program, the QTL can be localized within a confidence interval.

Two major difficulties challenge the users of QTL methodology. (a) A scan of the whole genome typically involves several markers on each of 20 independent chromosomes in mice, and in humans, 23. Thus, there is an appreciable risk of a false positive association when the conventional Type I error probability [Alpha] = 0.05 is used for each test, so most of the QTL harvest will probably be spurious. Lander & Krugylak (1995) argued persuasively that researchers should use [Alpha]= 0.0001 for each test to keep Type I error at 5% for the entire linkage study. (b) Even if the evidence for existence of a QTL is compelling, the width of the confidence interval along the chromosome may still be too great to allow for rapid gene identification and sequencing. A 1% recombination frequency corresponds to a distance along the chromosome of about 1 centiMorgan (cM), that in mice contains about 2 Mb of DNA and about 65 genes. A review of 22 QTLs believed to be important for alcohol and drug sensitivity (Crabbe et al 1998) found that the interval in most cases was more than 15 cm.

If the QTL can be localized only within a 15 cM interval, it could be any one of about 1000 genes (Belknap et al 1997).

Many claims of QTLs assigned to map locations have now been published in the behavioral genetics literature, and in some cases provisional gene symbols have been proposed. In many cases, the validity of these claims is suspect and the field would benefit from greater circumspection and rigor. Crusio (1998) remarks that "on closer examination, as yet the promise of the QTL method has not been fulfilled at all." It makes good sense to reduce Type II errors by casting a wide net in the first phase of a study, but it seems unwise to claim something has been mapped or provisionally mapped merely because there is statistically significant evidence of linkage. Further confirmatory testing should be mandatory to cull the false positives and substantially narrow the confidence interval (Darvasi 1998). Real success should be recognized not in long lists of weakly substantiated QTLs but in one or two conclusive discoveries of genetic variants with moderate effects.

Several fruitful strategies for confirming hypothetical QTLs are available (see Crabbe et al 1998, Darvasi 1998). Buck and coworkers (1997) studied severity of alcohol withdrawal symptoms in mice derived from the strains C57BL/6J and DBA/2J. An initial screening against 1522 genetic markers in 21 recombinant inbred (RI) strains yielded seven chromosomal regions appropriately designated as showing "potential linkage" with a "putative QTL." In a sample of 451 [F.sub.2] hybrid mice evaluated only at regions implicated in the first phase, three of these were clearly supported and another weakly supported (see Belknap et al 1996). The researchers then selectively bred two lines of mice for high and low withdrawal severity, and the allele frequencies at three marker loci diverged rapidly and significantly, thereby confirming the existence of three QTLs with independent evidence in the predicted direction. Although map locations suggested plausible candidate genes--including several GABA receptor subunits on chromosome 11--95% confidence intervals were more than 10 cM wide.

A similar approach has been employed to study the acute response (loss of righting reflex) to a high dose of ethanol. When 124 markers were tested in 27 RI strains derived from the long sleep (LS) and short sleep (SS) lines, 11 "provisional QTLs" were located with a very lenient ([Alpha] = 0.05) criterion (Markel et al 1996). A study of the mice with the most extreme scores in a sample of 1072 [F.sub.2] hybrids supported only two of these QTLs, which were tentatively localized within intervals of about 16 cM (Markel et al 1997). As a further test, [F.sub.2] hybrid mice of known genotype at the marker loci flanking the hypothetical QTL were then mated and their offspring tested for ethanol-induced sleeping (Bennett et al 1997). Although the sample sizes were too small to yield conclusive results, this application of marker-assisted selection (Ruane & Colleau 1995) holds great promise for confirming the presence of a QTL, localizing it to a narrower interval, and studying interlocus interactions.

Once the presence of a QTL has been adequately confirmed, its precise identity must be demonstrated. This is probably feasible only for a gene already documented at the biochemical level. From a chromosome map, researchers can locate plausible candidate genes in the confidence interval for their QTL. For example, Buck and colleagues (1997) noted that a QTL on chromosome 11 was near genes for three subunits of the GABA receptor (Gabra1, Gabra6, Gabrg2). The full DNA sequences of the exons of a gene in the two strains might reveal a polymorphism that gives rise to different forms of the protein. If only one of several candidate genes differs between the strains, it will become the object of intense scrutiny, whereas the presence of several polymorphic genes in the interval will confound progress. Further evidence could be obtained by knocking out the gene in question, but this evidence could also be misleading. The QTL itself might involve a rather minor difference in viable alleles, whereas a total knockout of another nearby gene might very well have major pleiotropic effects on that behavior. Thus, the knockout could implicate one gene without proving that gene to be the source of the QTL.

The task of identifying genes of moderate effect will benefit from a comprehensive effort in a wide variety of common mouse strains to determine the DNA sequence of exons for genes known to code for many nervous system proteins. Genes already proven with the knockout method to be relevant for brain development or

behavior would provide a good starting place. To date, the knockout method has taught us much about development but not about individual differences. The classical era of mouse behavioral genetics documented large variations among common inbred strains for a wide range of behavioral phenotypes. We need to know whether the genes targeted by molecular biologists are indeed the genes that gave rise to these ubiquitous strain differences. If researchers would assess the possible relevance for behavior of definite protein polymorphisms rather than search for the proverbial needle in the haystack using the QTL method, answers would come more easily and be less prone to error.

Allele Association Studies

The allele association approach is being used to assess the relevance of well-known nervous system proteins to behavioral variation in humans. In the first step, several alleles of a gene that lead to altered forms of a protein are identified. For example, a 48-base sequence coding for a string of 16 amino acids in the dopamine type 4 receptor (the DRD4 gene) is often repeated, and a world wide survey identified 9 alleles with 2 to 10 repeats (Chang et al 1996). Many relatively common alleles in the dopamine D2 receptor (DRD2) gene are also well documented (Kidd et al 1996). Of critical importance is the observation that allele frequencies generally differ markedly from one geographic population to another (Kidd 1996).

The second step is to establish a correlation between specific alleles and behavioral differences. Claims have been made--but doubts persist--that the A1 allele of the DRD2 gene leads to higher risk of alcoholism. If the study sample is ethnically diverse, an allele that is more common in a group which has a higher rate of alcoholism could result in a spurious correlation. The best recourse is to examine allele associations within a more homogeneous population. For example, in three populations in Taiwan, there is no association of alcoholism with alleles in either the DRD2 or DRD4 gene (Chang et al 1997, Lu et al 1996). In surveying the literature, Kidd (1996) concluded that "the better designed studies have been consistently negative on association" with alcoholism.

Genetic polymorphisms also speak to the chronically vexatious issue of race in behavioral genetics. A comprehensive assessment of allele frequencies around the world by Cavalli-Sforza and colleagues (1994) found little support for racial categories. More recent data prompted Kidd (1996) to comment: "It is my belief that racial classifications of humans are scientifically indefensible since there are essentially no boundaries of qualitative genetic difference and the vast majority of genetic variation shows a continuous pattern around the world."

Considerable publicity has been given to two studies published in 1996 that claimed an association between the personality trait of novelty seeking and the long 7 repeat allele of the DRD4 gene. As revealed in Figure 1, eight subsequent studies from several countries have obtained mixed results. A meta-analysis of these data suggests that scores on the novelty seeking questionnaire have a standard deviation roughly $d = 0.06$ higher in people with longer repeat alleles (95% confidence interval from -0.03 to 0.16). Because there is significant heterogeneity among the samples ($Q = 32.9$, $df = 9$, $P = 0.0001$), it is possible that epistatic interaction with the genetic background or interaction with test situations or local environments could yield a significant association in certain populations but not others.

[Figure 1 ILLUSTRATION OMITTED]

This exercise with meta-analysis and the history of false positive linkage results for schizophrenia teach important lessons. When a new claim is made of weak allele association or linkage with some other measure (such as IQ), experience should caution us against premature enthusiasm until the result is replicated adequately and survives meta-analysis. Otherwise, there arises a serious risk that false claims will mislead public discourse, as allegations of sex-based differences in the human brain (Bishop & Wahlsten 1997) and an alleged relation between serotonin metabolism and impulsive violence (Balaban et al 1996) have already done.

Presuming the allele association method does eventually point to a genetic variant with reliable behavioral correlates, proof that the connection is causal does not follow automatically. The one gene might be linked to another locus that is actually responsible for the observed difference, and hence it would be wise to assess several nearby genes rather than to restrict the scope of the search too early on. The knockout method will not, of course, be available for confirmatory studies in humans, but highly specific DNA-based drugs might be used to substantiate an effect on behavior for the locus in question.

Linkage and allele association methods are entirely adequate for detecting genes with large phenotypic effects, but these kinds of genetic variants tend to be uncommon in the human population. Nevertheless, the work can be justified because of the potential benefit new knowledge may provide for the prevention or alleviation of suffering. Meanwhile, the hunt for ubiquitous polygenes pursues an elusive quarry. Detecting small effects requires extraordinarily large samples, even if the best available research designs are employed (Risch & Merikangas 1996). There is a profound conflict inherent in this enterprise. In terms of a social calculus of the cost-benefit ratio, the smaller the potential good that might result from a new discovery, the more expensive the purchase of that knowledge will be.

Databases of Genetic Information

Despite my reservations about research on genes of small effect, impressive progress has been made in the detection of genes affecting the nervous system and behavior; and even a cursory account of the present state of knowledge exceeds the scope of this review. Fortunately, a vast reservoir of current genetic information is now readily available on the World Wide Web at species-specific sites (Table 1). Investigators can search these databases for long lists of genes residing on a specific chromosome, detailed information about a specific gene, or lists of genes with possible relevance to a specified phenotype or syndrome. A formal course on skills for the Web would be a very useful addition to the neurobehavioral genetics curriculum.

The Mouse Genome Database (MGD) can be reached via the Jackson Laboratory (jax) site. In February of 1998, a search for the phenotype "obesity" yielded 13 relevant genes. The gene symbol Lep, or its name, leptin (formerly obese), yielded the precise map location on chromosome 6, a lengthy abstract, a current bibliography, and other useful information. It also provided links to the homologous gene LEP in humans and the DNA sequence of several ESTs. If one does not know the official gene symbol, it is best to start with a search for a closely related keyword. For example, the calmodulin kinase II [Alpha] subunit gene symbol is Camk2a. If the protein symbol CaMKII (often cited in the neuroscience literature) is entered in a search of the MGD, nothing is found, whereas a search using the keyword "calmodulin" successfully calls up information on the gene in question and several others. The MGD currently lists 20,080 genetic markers that have been placed on the mouse chromosome map and 8911 genes, of which 6171 have been mapped and 6396 have at least partial DNA sequences available. One must exercise caution when searching for genes affecting phenotypes, because many in the database are poorly validated and may be ancient apparitions. One such is the gene absent corpus callosum (ac), originally reported by Keeler (1933) but not seen by anyone in the past 60 years. Once a gene is listed in the catalog--no matter how flimsy the case for its existence--it tends to remain there. Any mouse gene for which an accurate map position is lacking should not be taken seriously.

After completing a brief registration procedure on the BioMedNet Web site, one can access the Mouse Knockout Database, which provides extensive data on targeted mutations. A search detected over 300 articles on over 100 single-gene knockouts that yield viable animals with alterations in the nervous system and/or behavior.

The Weizmann Institute of Science Gene Cards facility is especially recommended for accessing human genetics information. It allows searches for gene symbols or keywords involving phenotypes, yields chromosome map locations, protein characteristics, and homologies with mice, and offers convenient connections to the Genome Database (GDB) or Medline literature search. The GDB is presently the most authoritative source on human genetics, but it may soon cease operations because funding by the US Department of Energy is being discontinued (Letovsky 1998). Online Mendelian Inheritance in Man(OMIM)

provides a lengthy abstract and bibliography for each gene and can also be accessed by entering phenotypic keywords. Searching OMIM for "dyslexia" in February 1998 yielded three gene symbols (DYX1, DYX2, THRB). The GDB listing for DYX1 is based on a single entry from July 19, 1996, and the existence of this gene is far from certain; no information is cited on chromosome map location, and the fine print reveals that it is merely a "reserved symbol," meaning that this will be its official designation if the gene is ever confirmed. DYX2 yields a map location on chromosome 6 that has been supported by an independent group of researchers (Grigorenko et al 1997) but only for one of five reading-related phenotypes (phonological awareness) and only with nonparametric (rather than parametric) methods. The confidence interval for gene location is more than 10 cM wide, and no protein or DNA sequence information is known. A search on "schizzophrenia" yielded 40 entries including the gene symbol SCZD1 assigned on April 12, 1989, to a region on chromosome 5 that is now recognized as not harboring a gene influencing schizophrenia (Moldin & Gottesman 1997). Any gene name returned by a search of OMIM should be carefully checked against more authoritative sources, especially the GDB, where a history of the SCZD1 symbol reveals it was "unassigned" on October 16, 1991. If a protein, DNA sequence, or homology with a mouse gene is listed, one can be confident that the gene is real, but a map location by itself provides no guarantee. Several symbols included in the catalog represent false positives that have not been culled.

The only unequivocal evidence for a gene is elucidation of its DNA sequence and associated protein structure. The quality control for this kind of biochemical information on the Web is good, in part because of facilities provided by the Human Genome Project. Unfortunately, quality control for weaker claims about genes relevant to phenotypes is inadequate, and speculative assertions in the mass media about genetic determination of socially significant behaviors (Colt & Hollister 1998) all too often are based on hasty proclamations from behavioral geneticists who should know better.

GENE FUNCTION

A cornucopia of genes relevant to the nervous system and behavior is now available for research on function. The question of how genes influence behavior and how the activities of genes are themselves regulated is of prime concern for psychology. Function can be understood at different levels.

Natural Polymorphisms

A mutation that seriously impairs the function of an important gene typically is rare in a breeding population, but not all major gene effects on behavior are grossly aberrant misfits. Two remarkable behavioral polymorphisms found in wild fruit flies seem to persist because they aid a species to exploit a wider variety of environments. The foraging locus influences activity of larvae in the presence of food; the dominant rover allele ([for.sup.R]) leads to longer forays into the environment, whereas the recessive sitter allele ([for.sup.s]) results in more localized feeding. Both alleles are common in wild fruit flies living in an urban habitat (Toronto). An exemplary series of studies demonstrated that the sitter mutation occurs in a previously documented gene, dg2, that codes for a cyclic GMP-dependent protein kinase and causes a small change in activity of the enzyme that is sufficient to alter foraging behavior (Osborne et al 1997). The rover phenotype predominates in crowded living conditions, whereas the sitter allele increases in lower population densities where the food supply is not so readily exhausted (Sokolowski et al 1997). A more subtle polymorphism occurs in the circadian clock gene period, where the allele which is more common in northern Europe leads to more efficient adaptation of the 24-h activity rhythm to temperature changes than the allele more common near the Mediterranean (Sawyer et al 1997). By combining carefully controlled genetic analysis in the laboratory with studies further afield, the science of individual differences thus advances our understanding of behavioral ecology and evolution.

Genetic Dissection

Most psychologically interesting behaviors are multifactorial, involving numerous genes whose actions are influenced by diverse features of the environment. Although individual studies usually concentrate attention on one specific gene, it is generally understood that many genes are relevant. As emphasized by Tully (1997), "Single-gene mutant analysis can be informative only when pursued within the framework of interacting polygenes." Powerful techniques to create mutations have spawned new possibilities for genetic dissection of

complex processes. No satisfying account of genetic involvement in any complex behavior has yet been achieved, but significant progress has been made in several domains. Olfactory learning and memory in fruit flies is a process in which certain mutations exert their effects primarily on a specific component (see Figure 2), but the famous flow diagram does not imply that one or two genes provide a sufficient explanation for a biologically distinct component of memory; yet the diagram is a useful device for integrating a large corpus of experimental data. The fact that different mutations result in flies with different temporal profiles of memory loss and different interactions with drugs that block protein synthesis proves the multifactorial nature of the memory process. By examining flies affected simultaneously by two different mutations, a scheme for parts arranged in series or in parallel may be perceived. Numerous other genes are undoubtedly involved, and pleiotropy, the occurrence of multiple phenotypic effects of one gene, is to be expected. For example, the turnip mutation reduces motor activity and sensitivity to shock while also impairing learning (Mihalek et al 1997). To some readers, this may render it less interesting because its effects are not restricted to the memory process, but genetic dissection clearly reveals it to be an integral part of the process.

[Figure 2 ILLUSTRATION OMITTED]

Targeted mutations have led to a resurgence of interest in learning and memory in mice, and the list of genes known to be important is rapidly growing (see Table 2). Although admirable efforts have been made to comprehend the interconnections of gene-derived proteins involved in memory formation within a synapse (e.g. Abel et al 1998), the horizons of this metabolic landscape are rapidly expanding, with no limit in sight. Many of these genes have pleiotropic effects as well, such as Camk2a, which is important in spatial memory but also impinges on numerous other behaviors (Chen et al 1994), and Creb, which also reduces symptoms of morphine withdrawal (Maldonado et al 1996).

Table 2 ***Mouse genes on specified chromosomes that are important for psychological processes(a)***

Chromosome	Learning and memory		
1	Creb1	Sele	
2	dbh	Grin 1	
3	Gria2	112	
4	Pde4b		
5	Ache	En2	Hdh
6	Kcna1		
7	Pkcc		
8	Pkaca		
9	Ncam	Rasgrf1	
10	Fyn		
11	Adcy1	Cbx2	
12	Fos		
16	App		
18	Camk2a		
X	Fmr1		

Chromosome	Appetite and obesity			
1				
2	[A.sup.Y]	anx	Mc3r	
3	Ap2			
4	[Lepr.sup.db]			
5				
6	[Lep.sup.ob]			
7	Ad	Gys1	tub	
8	[Cpe.sup.fat]	Insr	Mclr	Mt1
9	Cck			
10	Adn			
11	Slc2a4			

12	Pomc1
16	
18	
X	Htr2c

(a) Genes included in the table must have an accurate map location in the Mouse Genome Database and be implicated by at least one study in the process. Most were demonstrated by the targeted mutation method, although a few were spontaneous mutations. Superscript symbols refer to the neurological mutations known prior to the discovery of the specific protein for which the gene codes. Human homologues have been verified for all but four genes in the table.

The organism's genes are of course present from conception, and many participate in formative processes as well as in dynamic adult functions. Embryonic effects can be notably different from involvement in the mature brain, and to distinguish between developmental and current effects of a gene knockout is challenging indeed. Several clever techniques have been employed to overcome this problem. Tsien and coworkers (1996) deleted the NMDA receptor (Grin1 gene) selectively from the CA1 region of the hippocampus and obtained memory deficits similar to those from a nonspecific gene knockout; Mayford and colleagues (1996) were able to limit the expression of a Camk2a mutation to the forebrain of adult mice and still obtained memory deficits. Guzowski & McGaugh (1997) altered spatial memory by injecting synthetic DNA directly into the hippocampus of adult rats to specifically modify the action of the Creb gene. These sophisticated methods confer unprecedented clarity on results for psychopharmacology. Molecular genetics is thus becoming a tool in the kit of physiological psychology.

Appetite and obesity in mice are proving to be physiologically and genetically complex (Table 2), and conceptual schemes for synthesizing this knowledge are still lagging behind the burgeoning data. This is happening with regard to circadian rhythms as well, where newly discovered genes are revealing previously unimagined elements of a larger picture (Albrecht et al 1997).

Investigations of obesity provide an emerging portrait of diverse organs connected in feedback loops involving the environment (Figure 3). Under normal conditions, overeating leads to growth of white fat cells (adipocytes) which in turn synthesize the protein leptin and secrete it into the bloodstream. One of leptin's effects occurs in the hypothalamus, where it binds to the leptin receptor and decreases appetite by inhibiting the synthesis of neuropeptide Y (Npy gene)--a neurotransmitter that tends to increase appetite. The obese mutation ([Lep.sup.ob/ob]) prevents the synthesis of leptin in white fat, thereby increasing appetite when NPY levels rise unchecked, and gene therapy to restore leptin in the [Lep.sup.ob/ob] mice prevents both obesity and diabetes (Muzzin et al 1996). The diabetes mutation ([Lepr.sup.db/db]) disables the leptin receptor and renders mice insensitive to high levels of leptin in the blood, which again leads to overeating (Caro et al 1996). By using a double mutant combining [Lep.sup.ob/ob] with the gene knockout [Npy.sup.-/-], it was shown that there are parallel pathways for leptin-related appetite control in the hypothalamus (Erickson et al 1996). The [Lep.sup.ob/ob] plus lethal yellow ([A.sup.Y]/a) double mutant revealed another parallel pathway that acts via the melanocortin-4 receptor (Mc4r gene) where normal stimulation of MC4-R decreases appetite but the [A.sup.y] gene product antagonizes it (Boston et al 1997). Not all obesity is mediated by the leptin loop (Schonfeld-Warden & Warden 1997), not all leptin effects are mediated by appetite changes (Yu et al 1997), and diabetic symptoms are not joined inexorably with obesity (Hotamisligil et al 1996). Although Table 2 suggests that separate sets of genes impact learning and appetite, this inference may not be warranted because those working with obesity typically do not assess a wide range of behavioral phenotypes. It seems highly likely that variations in appetite would indeed influence the acquisition of certain kinds of tasks, but little recent work with obesity mutations has been done by psychologists interested in motivation.

[Figure 3 ILLUSTRATION OMITTED]

Genetic dissection thus proceeds through several stages. (a) Research projects in the early stages seek to discover a single mutation and explore its phenotypic effects. (b) After several genes are known to be important

parts of the system, work begins with double mutants and factorial gene-environment or gene-drug interaction studies to elucidate serial and parallel processes, each study focusing on a limited sector of the larger system. (c) Eventually, attempts are made to integrate this knowledge into comprehensive models that can be tested with multifactorial experiments. Most research in neurobehavioral genetics is presently entering the second stage, and none has yet reached the third.

Systems of Genes

The question remains of how many genes are involved in memory, appetite, or circadian rhythm. A first approximation can be achieved by examining the array of genes expressed in mRNA under specified conditions. A sensitive and rapid method is now available to assess simultaneously the expression of hundreds of genes in mice (Figure 4) and over 1000 in humans, and customized arrays for screening any desired subset of genes may be anticipated (see Web sites www.resgen.com and atlas.clontech.com). One might contrast brains of trained and untrained mice to assess memory, or brains at midnight and at high noon under a normal light cycle or constant darkness to reveal circadian mechanisms. Furthermore, tissue from mutants and normal siblings tested under the same circumstances could be used to assess pleiotropic effects.

[Figure 4 ILLUSTRATION OMITTED]

An extraordinary glimpse of complex gene action has been obtained recently for yeast, an organism best known to psychologists for its vital role in synthesizing ethanol from sugar. As the sugar in the yeast's environment is consumed, its metabolism shifts from anerobic fermentation to aerobic respiration. Researchers were able to attach DNA sequences of almost all the 6297 yeast genes to a single glass plate 18 mm by 18 mm and record the abundance of all mynas at different stages of the metabolic process (DeRisi et al 1997). During the transition from anerobic to aerobic metabolism, the expression of 1740 genes increased or decreased at least twofold. About half of these genes were new to science, had not yet been named, and had no recognized functions. A mutation in a single gene (tup1) altered the expression of 355 other genes. In a remarkable understatement, the authors observed: "The large number of genes whose expression is altered and the diversity of temporal expression profiles highlighted the challenge of understanding the underlying regulatory mechanisms."

The one-celled yeast, of course, is a relatively simple creature that has been thoroughly studied since the time of Pasteur. It seems likely that the complete picture of gene activity during mammalian learning and memory will be even more complex. The molecular tools are close at hand but the prevailing conceptual framework in biological psychology may not be equal to the task of integrating so vast an array of data.

DNA analysis and gene discovery have been dominated by a very successful reductionistic perspective (Beckwith 1996), but research on gene function reveals the necessity of systems-oriented thinking (Gottlieb et al 1998, Strohman 1997). The idea that a gene determines a specific component of a behavioral phenotype is losing scientific credibility. Chenchik and coworkers (1998) forsee that new methods "will lead researchers away from reductionistic approaches which focus on single genes, and towards more systemic approaches that involve the simultaneous, parallel analysis of hundreds or thousands of genes." It must be acknowledged that almost every gene has widespread pleiotropic effects (Miklos & Rubin 1996), that actions of genes are commonly altered by the organism's environment (Gottlieb 1998), and that the consequences of a specific mutation often depend on genotypes at other loci (Varnam et al 1996) and the genetic background (de Belle & Heisenberg 1996, Kelly et al 1998, Miklos & Rubin 1996) as well as on epigenetic effects (Wolf 1997). Strohman (1997) concludes that the origins of complex systems "are not to be found in the matter itself, but in its interactions."

BEHAVIORAL TESTING

Spectacular advances in genetic analysis have captured the imagination of the public and drawn legions of students into molecular biology. The effect has not been to impoverish psychology but to renew interest in the psychology of behavioral testing--especially regarding lab mice--as testified to by the 1996 Society for

Neuroscience short course entitled "What's wrong with my mouse?" (Takahashi 1996). Specialists in behavioral genetics have been both impressed by the enthusiasm and appalled at the naivete of molecular geneticists who believe psychology can provide an off-the-shelf device to measure a specific construct in mice and model its human counterpart. The new molecular genetics has created a need for a wider variety of behavioral testing and for improved test construction and standardization. The skills of psychologists are uniquely suited to this task.

Interactions with Test Situation and Environment

There is a rich variety of tests available for use with mice (Crawley et al 1997, Crawley & Paylor 1997), and it is important to know whether these are likely to yield the same results in the hands of most investigators working with the same strain or mutation. The test situation and the pretest environment are virtually never the same in different laboratories. The central issue is therefore whether genetic and environmental effects are additive or interactive. If additive, then differences among labs will merely change the overall average score but will not alter the pattern of results or rank orders of genotypes, and most tests should yield valid results even in the hands of amateurs.

A clear answer can be provided to this question. Seemingly minor task-specific factors interact strongly with genotype, and reversals of rank orders of strains are commonplace when comparing results across labs. Recent work has emphasized the importance of relatively subtle variations in protocols. Poderycki and coworkers (1998) evaluated hybrid crosses of mice for seizures induced by 5-15 repetitions of gentle tossing. Genetic analysis revealed strong evidence of linkage with a marker on chromosome 9 after 6 tests but not after 15 tests, whereas another gene on chromosome 2 was not apparent after 6 tests but showed clear signs of linkage after 15 tests. Maxson (1992) reported that the effects of the Y chromosome on agonistic behavior in his congenic strains disappeared when the colony was moved to a cleaner environment where the drinking water was acidified to suppress bacteria. Certain Y chromosome effects were most pronounced when males were reared in isolation and tested against males of the same genotype, rather than reared with a sister and tested against a standard opponent strain (Guillot et al 1995). Peeler (1995) conducted avoidance training at different times of day, all during the light phase of the cycle, and found substantial effects on some strains but not others.

Apparatus design and testing protocol are crucially important. Roullet and colleagues (1993) found that BALB/c mice used odor cues and C57BL/6 mice used spatial cues to learn a radial maze, whereas [F.sub.1] hybrids could utilize either cue. Crusio and coworkers (1993) found large changes in strain rank orders on spatial versus nonspatial versions of a radial maze; only the spatial version revealed genetic correlations with hippocampal mossy fiber anatomy. Peeler (1995) noted differences in strain rank order depending on whether the mice had to run through a hole or slot or jump a barrier to avoid shock. These and other findings show clearly that a single test configuration and procedure cannot define a single psychological construct, although two tests differing in a specific element may indicate a change in a specific construct.

Growing Need for Test Standardization

In view of these findings, there are grounds for concern about the almost universal lack of standardized apparatuses, protocols, and lab environments in psychological testing of animals. In contrast, test construction and standardization are taken more seriously in evaluation of humans. Reviews among labs of the plus-maze, a popular means for assessing anxiety, reveal numerous idiosyncratic variations that are potentially important (Hogg 1996; Rodgers & Dalvi 1997). Tests known to be valid for rats are often used inappropriately with mice. The Morris swimming pool is particularly problematic when used with mice (Whishaw & Tomie 1996). Some common inbred strains (BALB/c, 129) respond quite badly in this device (Francis et al 1995) and often resort to floating after becoming exhausted (Wolfer et al 1997). When mice can locate a submerged platform, they must be using spatial cues, but failure to learn does not necessarily signal a lack of spatial memory (unless only the cues are manipulated in different versions of the task, and a strain can learn one cue but not the other). Additional difficulties are present when investigators submit an individual animal to a battery of tests, each of which was designed and validated for use by itself: Order of testing can markedly alter results when such tests are combined.

Validity of Animal Models

Whereas homology among flies, mice, and humans at the molecular genetic level is undeniable, homology--and even analogy--at the level of the behaving organism is not so clear. Certain genes important for memory in flies (Figure 2) are involved in memory in mammals (e.g. the fly gene rutabaga, the mouse gene barrelless, and the human gene ADCY1 all encode an adenylate cyclase), yet the complex nature of metabolic and developmental systems (characterized by pleiotropic and epistatic gene actions plus gene-environment interactions) implies that the function of a particular gene depends on its context. Homology of behaviors must be demonstrated, not assumed. In the domain of agonistic behavior, for example, mice engage in offensive and defensive attacks that appear to be adaptive under appropriate circumstances, but do these provide good models of human violence? According to Maxson (1998), offensive behavior of male mice is not a good model of impulsive aggression in humans, although several recent publications assume uncritically that the two are essentially the same. Balaban and colleagues (1996) also stress the importance of careful definition of behaviors and contexts when seeking to establish the relevance to humans of animal models, and they question the similarity of rodent attack behavior to human crime. A good case can be made for valid mouse models of several severe medical disorders caused by single-gene mutations, but the validity of mouse or fly models for the normal range of variation in human social behavior requires convincing evidence that is generally lacking.

CONCLUSIONS

In his review of human behavioral genetics, Rose (1995) foresaw that "Future reviews of the field are likely to read very differently than this one." The field has indeed changed direction and is advancing like a sailboat with spinnaker unfurled, rather than tacking and making little headway. Many outstanding contributions to neurobehavioral genetics are now published in leading scientific journals with a broad readership rather than in specialty journals. Not long ago, the field was trammeled by crude techniques for detecting the presence and activities of single genes, whereas today we have a panoply of molecular methods and a rich factual base of knowledge about specific genes in relation to brain and behavior. Long lists of human attributes, each accompanied by a terse summary of the latest findings from twin or adoption studies, have become passe. The challenge of keeping aware of current developments in this field is now quite formidable, even with the aid of marvelous Internet and bibliographic search programs. As the individual research project probes ever more deeply into an ever-narrower domain of knowledge, there is a growing need to synthesize existing knowledge and make connections among the isolated parts of an expanding discipline. The next major advance must come in the domain of theory.

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