GENETIC CONTRIBUTIONS TO ADDICTION*

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■ **Abstract**   Even the most extreme environmentalists along the nature-nurture con-
tinuum in psychology now acknowledge that genes often contribute to individual dif-
ferences in behavior. Behavioral traits are complex, reflecting the aggregate effects of
many genes. These genetic effects are interactive, *inter se* and with the environments
in which they are expressed. Human studies of addictive behaviors have clearly impli-
cated both environmental and genetic influences. This review selects drug dependence
as a paradigmatic addiction, and further, concentrates on the extensive literature with
genetic animal models. Both traditional studies with inbred strains and selected lines
and studies exploiting the new molecularly based technologies of the genomics era are
discussed. Future directions for further contribution of animal models studies to our
understanding of the brain dysregulations characteristic of addictions are identified.

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DEFINING ADDICTION

Addiction is a lay term, so everyone assumes he or she knows what it means. However, serious discussions of the basis for the motivational dysregulation of behavior that is its core feature must navigate between languages seeking to describe physiological/pharmacological sources of influence and those focused on intrapsychic events whose basis is unspecified. An interesting review of the field that evaluated this distinction forthrightly concluded that both orientations contribute to our understanding of addiction, and that both structural frameworks can offer predictive value (Davies 1998). This review takes up the recent neurobiological evidence. In addition, it focuses on genetic animal models, which have contributed much of our knowledge of addiction biology.

Which behaviors fall into the category of "addiction" is a matter of debate, but genetic studies have helped in some cases to clarify the issues. For example, whereas all would agree that obesity has a behavioral component, the multiple potential sources of dysregulation that lead to obesity (e.g., in appetite, overeating, and physiological processing of foods) can support multiple opinions about the essential pathology. Recent animal studies have identified multiple genes influencing each of these component processes. A review of these individual genes' effects in mice demonstrates that we still cannot explain all forms of obesity simply with these genes, but there are specific behaviors and/or physiological events affected by each (Wahlsten 1999). Armed with this knowledge, we can more readily assess the extent to which certain cases of obesity may represent addiction.

Most would agree that overuse of alcohol and other abused drugs represent clear examples of addictions. Although other addictive behaviors are occasionally discussed, this review concentrates on drug addiction for three principal reasons. First, there is a wealth of data on the genetics of drug dependence. Second, many of the key features of addiction have been modeled successfully in mice and rats. Finally, drugs can be studied from the framework provided by their pharmacology. Drug receptors are localized in the brain, and drug effects are often local to specific brain regions. Specific tools (e.g., antagonist drugs) are often available. Furthermore, the experimenter can arrange access to and delivery of drugs to the subject. These and other specific features of drug dependence, coupled with a population incidence in US adult males diagnosed as alcoholic and/or drug dependent of more than 10%, have led to intense study in this area.

To understand the genetics of individual differences in susceptibility to abuse drugs, we need to consider several aspects of drug response to be comprehensive, including (a) susceptibility to an initial challenge with a drug; (b) neuroadaptation that occurs with chronic drug administration, represented as reduced (tolerance) or increased (sensitization) sensitivity; (c) dependence, as inferred from the presence of withdrawal symptoms when the drug is removed; (d) the reinforcing effects of the drug, which may be positive or negative, and in humans are often characterized as craving; and (e) the efficiency of metabolism and elimination of the drug. In addition to these issues, drug studies have wrestled with modeling presumptively

important, related neurobiological factors such as impulsivity/disinhibition/ loss of control, and various neurobiological and behavioral aspects of stress responses.

The neurobiological bases of drug addiction have stimulated several theoretical approaches. Most evidence historically has centered on explanations that seek to identify dysregulation in the brain circuits underlying reward more generally, primarily in the dopamine systems of the basal forebrain. The various approaches and neurobiological studies testing these hypotheses are reviewed elsewhere (Wise 1996, Self & Nestler 1995, Altman et al. 1996, O'Brien et al. 1998, Robbins & Everitt 1999, Freeman et al. 2001, van Ree et al. 1999, Tzschentke 1998). Genetic strategies have provided much important evidence (Nestler 2000): Before taking up the genetic evidence, a few terms and concepts are introduced. More description can also be found in a recent review of behavior genetics (Wahlsten 1999).

GENETIC APPROACHES TO ADDICTION

Genetics and Genomics

Even the most extreme environmentalists along the nature-nurture continuum in psychology now acknowledge that genes often contribute to individual differences in behavior. No reasonable person would argue that genes determine behavioral outcomes, but understanding their influence is important. Each individual possesses two alleles at each gene, one inherited from each parent. When different alleles at a gene are circulating in a population, the gene is said to be polymorphic, and these different alleles are represented as differences in the base sequence of the DNA coding for the gene product, a protein. So, the first level of genetic variation that can give rise to individual behavioral differences is due to DNA sequence differences.

However, complexity is introduced by several other aspects of gene structure and function. A gene directs production of a protein via the intermediary molecule, RNA. When an RNA is synthesized, or transcribed, many DNA sequences (called introns) are ignored, and the RNA (which will be translated into protein) represents only some of the original genomic DNA, specifically that from the "coding regions" (exons). However, some DNAs lead to multiple splice variants of RNA, hence to multiple proteins. Furthermore, all cells contain identical DNA sequences, but the genes are not expressed all the time. Expression of each gene is regulated differentially among body tissues and organs, at developmental periods throughout life, and even in different regions of the brain. Some genes are not expressed at all in certain tissues.

Finally, behavioral traits are complex. They are rarely affected by only a single gene. Indeed, they have been characterized as multigenic or polygenic in recognition of the fact that any given gene is likely to contribute only a small influence on phenotypic (behavioral) variance. The complexity is also the result of pleiotropy, the term geneticists use for the impact of a single gene on multiple behavioral traits.

As discussed below, the ultimate effects of genes may prove to be very different in different environments.

The recent strides forward in our understanding of genetic influences on behavior have been elegantly reviewed (Wahlsten 1999), and some specific implications of the genomics revolution discussed (McGuffin et al. 2001). The principles of gene action sketched above apply equally to human and nonhuman animal studies. However, the methods for performing experiments and statistically analyzing outcomes are radically different. Because of the much greater experimental control over animal studies, our review concentrates on them. We also follow the lead of other recent reviews (Wahlsten 1999) and concentrate on the complex interactions among genetic and environmental conditions in an attempt to address the problem of addictions from a behavioral genomics perspective, i.e., one that emphasizes the effects of genes on behavioral functions of the whole organism (Plomin & Crabbe 2000). This perspective may be contrasted with the much more prevalent functional genomics and proteomics efforts to identify for each relevant gene the ways in which that gene's protein product affects cellular function.

Human Genetic Studies

TWIN/FAMILY/ADOPTION STUDIES   The classical approaches to complex trait genetics in psychology have been the examination of co-occurrence of or comorbidity for the trait in monozygotic vs dizygotic twins, reared together or apart, and in analogous family studies with other sorts of biological relatives. These studies, particularly when coupled with genetic epidemiological analyses (Merikangas & Swendsen 2001), have provided solid evidence of genetic influence on addictions. Discussions of the older human behavior genetic studies relating to addictions can be found in an earlier review that considers personality, cognitive, and a broad range of psychopathological traits in addition to the addictions (Rose 1995). This review also offers an excellent survey of the complex interpretive issues surrounding family and twin genetic studies, as well as many other helpful insights into such issues as shared versus nonshared environmental effects and the chronic misperception of genetic data by the popular press.

Recent twin studies have explored such issues as the nature of the environmental contributions to alcohol abuse and dependence (Prescott & Kendler 1999; van den Bree et al. 1998a; Johnson et al. 1996b, 1998), caffeine dependence (Kendler & Prescott 1999), and comorbidity of alcohol abuse and depression or other psychiatric disorders (Prescott et al. 2000, Pickens et al. 1995, van den Bree et al. 1998b). An enduring issue in the addiction literature is the potential role of genetic influences in the substantial comorbidity for abuse of alcohol and other drugs. Alcoholism and smoking are highly genetically comorbid (Rose 1995, Madden et al. 1999, Stallings et al. 1999), and alcoholism and drug dependence also share common genetic influences (Rose 1995; Pickens et al. 1991, 1995; Tsuang et al. 1996). However, each disorder also reflects independent genetic influences as well (Enoch & Goldman 2001).

An issue related to comorbidity is whether or not certain personality traits are risk factors for addictions, including substance abuse. This notion has a very long history (Marlatt et al. 1988), but has always faced serious criticism (Nathan 1988). A systematic attempt to identify "addictive" personality traits failed to do so (Rozin & Stoess 1993). Nonetheless, the extensive genetic comorbidity among addictive disorders contributes to the idea's longevity (Patton et al. 1994, Williams 1996). The basic problem in testing the hypothesis of an addictive personality type is that the addictions are diagnosed post hoc, making it difficult to ascribe personality characteristics to the cause or effect column of the ledger. For example, depressive symptoms in many alcoholics resolve after abstinence (Schuckit et al. 1997).

Another example is the potential relationships among pathological gambling, substance abuse disorders, and personality characteristics. A thoughtful review explored these issues and noted the comorbidity of gambling and drug abuse, but did not address potential genetic bases for this relationship (Murray 1993). More recent studies, including a twin study, have also noted this pattern of comorbidity, a notion supported by the *DSM-III-R* and *DSM-IV* criteria for problem gambling (Slutske et al. 2000). A recent review raised the possibility that genetic studies may in fact help to elucidate the nature of excessive gambling behavior and discussed the implications of characterizing problem gambling as an addiction (Shaffer 1999). All investigators agree that it shares with the addictive disorders the feature of diagnostic heterogeneity.

Finally, one set of genetic findings has proven to be especially intriguing. Those individuals with a positive family history for alcoholism have been known to display reduced sensitivity to certain of alcohol's acute effects, such as body sway and subjective intoxication, compared with those who are family-history negative. A cohort of young men was ascertained in the late 1970s and followed prospectively as they entered the age of maximum risk for alcoholism. Family history was expected to predict later risk of alcohol dependence and alcoholism, consistent with a genetic contribution. Family history was indeed found to be predictive of alcoholism in a follow-up 8 years later, but level of initial response to drug challenge was even more highly associated with later alcoholism, and initial responsiveness (which itself is likely to reflect genetic influences) appeared to account for most of the variability in susceptibility to alcoholism (Schuckit & Smith 1996, Schuckit 1999). Other biological correlates of genetic risk for alcoholism have been recently reviewed (Begleiter & Porjesz 1999, Schuckit 2000).

Twin and family studies will continue to contribute to our understanding of the genetic etiology of addictive behavior. However, such studies cannot easily provide evidence of either the number, location, or identity of the responsible genes unless coupled with molecular strategies described in the next section.

MOLECULAR GENE-FINDING METHODS: ASSOCIATION AND LINKAGE STUDIES   One approach to understanding genetic influences on addictions is to relate genetic markers (i.e., specific sequences of DNA in the genome) to the phenotype across individuals. A cogent introduction to these methods for the nonspecialist can be

found elsewhere (Gelernter 1999). Studies of this sort have provided a steady stream of reported localizations for candidate genes or for markers at particular genomic loci (see for example Foroud et al. 1998, 2000; Loh et al. 2000). Many alcoholism and substance abuse–related loci have been discussed elsewhere (Reich et al. 1999, Chen et al. 1999). The low sensitivity to alcohol's effects discussed above has recently been mapped to specific markers as well (Schuckit et al. 2001). One interesting recent study suggests that impulsive-aggressive alcoholics were identified by a marker near the serotonin 5-HT$_{1B}$ receptor gene in two populations (Lappalainen et al. 1998). Much neurobiological evidence links serotonin dysfunction, alcoholism, and aggression (Le Marquand et al. 1994a,b). Another study found evidence for quantitative trait loci associations common to both alcohol consumption and smoking (Bergen et al. 1999).

Although association and linkage methods can sometimes provide strong evidence that there must be a gene near the linked marker that affects the trait, the methods are inherently limited by relatively weak effects of specific genes. More importantly, false positive associations are common, largely because populations that appear genetically rather uniform may in fact show a great deal of variation in gene frequencies for genes in the associated region (Gelernter 1999). It is often difficult to avoid situations in which the control and addicted groups are in fact drawn from two genetically distinct populations, a condition called stratification. The transmission disequilibrium test and variants thereof can mitigate this difficulty somewhat. These methods compare marker frequencies among relatives within family groups who share the trait with those who do not. Because all family members are by definition drawn from the same population genetic stratum, differences in some markers but not others are less likely to represent false-positive associations (see Long & Langley 1999, Uhl 1999 for discussion).

In the end, even very strong association and linkage data for markers near the gene for Huntington's disease required many years of additional work before the gene itself was isolated (MacDonald et al. 1993), and this is a single-gene, virtually all-or-none disorder. Final proof that a candidate association has truly captured the relevant gene or genes requires a collection of converging evidence drawn from a wide range of genetic and nongenetic techniques (Belknap et al. 2001).

Genetic Animal Models

Compared to genetic studies of other areas of psychopathology, it is in the area of genetic animal model development and utilization that genetic studies of alcohol and substance addictions are the most advanced. This is due to two factors. First, serious attempts to study voluntary alcohol drinking in rats began in the late 1940s and were followed by studies in mice in the 1960s. These studies had an explicit genetic orientation that has been sustained ever since. The second factor was raised at the beginning of this chapter: Drugs can be administered systematically by the experimenter or the experimental animal, and pharmacology provides a theoretical framework within which attempts to understand their effects can be organized.

INBRED STRAINS   The simplest genetic animal model system is the study of existing genetic variation. In 1959 McClearn & Rodgers studied several inbred strains of mice by offering them a choice between a bottle filled with tap water and one containing alcohol. Same-sex members of an inbred strain are essentially genetic clones owing to many generations of brother-sister matings, which reduces allelic variation at each gene until it eventually disappears entirely. They found that the differences among strains in preference for ethanol far exceeded the within-strain differences (McClearn & Rodgers 1959). This demonstrates a significant genetic contribution to the trait, because differences among strains assessed in as invariant an environment as possible can only arise from the underlying genetic differences. In particular, C57BL/6-strain mice were high preferrers, whereas DBA/2-strain mice were nearly complete abstainers, and other strains showed intermediate preference.

This pioneering study has been followed by dozens of other studies comparing strains for alcohol and drug sensitivity, tolerance, dependence/withdrawal severity, and propensity to self-administer drugs (Crawley et al. 1997, Marks et al. 1989, Stitzel et al. 2000, Seale et al. 1984; for reviews, see Crabbe & Harris 1991, Mogil et al. 1996). A major advantage of the inbred strain work is that the genotypes remain stable over time: Studies of C57BL/6 and DBA/2 mice performed in the 1990s have been compared directly with those from the 1960s, and the result has been a rich accumulation of knowledge about a few strains of mice. A disadvantage, however, is that the specific genes responsible for the strain differences are anonymous. Nonetheless, comparisons among characteristic strain mean responses through correlational analysis have taught us much about codetermination of genetic influence. For example, mouse strains that are high alcohol preferrers tend to be those that show minimal withdrawal severity when the drug is removed. The genetic correlation between these responses has been estimated to be as high as $r = -0.65$ across 15 inbred strains (Metten et al. 1998). A similar analysis of results from 13 inbred strains of mice demonstrated that efficiency of response inhibition assessed in a signaled nosepoke task was highly predictive of low ethanol consumption (Logue et al. 1998). Together, these studies suggest that strains genetically predisposed to experience severe withdrawal and to be able to inhibit responding are those who elect not to self-administer alcohol when it is offered.

There are more than 100 inbred mouse strains available. A new initiative called the Mouse Phenome Project has been undertaken to support systematic collection of behavioral and physiological data in a number (up to 40) of inbred strains and is assembling a relational database for centralizing access to such genetic relationships (Paigen & Eppig 2000). There are also many rat inbred strains, but they are in general less systematically characterized for traits related to addiction.

SELECTED LINES   The oldest technique in behavioral genetics is that of artificial selection. By arranging matings such that extreme responders are mated, lines of mice or rats have been selected to differ genetically in sensitivity, tolerance, dependence, and preference for alcohol and several other drugs of abuse. The

principles were derived from agricultural genetics, in which crops and animals are bred for favorable traits. When such selected lines are compared for other traits, they are often found to differ. Ideally, this is because of the pleiotropic effects of the genes underlying the selected trait, but care must be taken to insure that the change in genes caused by the limited population sizes one can actually maintain are not the cause of the differences in correlated responses to selection. Such studies have been of immeasurable value in advancing our understanding of the neurobiological basis for individual differences in drug responses.

The first selected lines relevant to addictions were developed in Chile in the late 1940s, where UChB rats were bred for high and UChA rats for low alcohol preference (Mardones & Segovia-Riquelme 1983). Mardones' studies, and the identification of the propensity of C57BL/6 inbred mice to prefer drinking alcohol solutions mentioned above (McClearn & Rodgers 1959), stimulated the first modern systematic studies of genetic determinants of alcohol and drug responsiveness.

Studies with these selected lines have been reviewed (Crabbe & Li 1995, Eriksson 1972, Crabbe et al. 1994, Li et al. 1994). Most lines have been selected for responses to alcohol, but selection has also been applied for opioid drugs (Mogil et al. 1995, Belknap et al. 1983), nicotine (Schechter et al. 1995), and cocaine (Marley et al. 1998), among other drugs (for reviews, see chapters in Crabbe & Harris 1991, Mogil et al. 1995, Mohammed 2000).

Perhaps the best known of these selected lines are the Preferring and Nonpreferring lines of rats and two additional pairs of lines subsequently derived for the same alcohol preference trait, High Alcohol-Drinking and Low Alcohol-Drinking rats. Many correlated responses have emerged that differentiate these animals, and only a few highlights are summarized here. Under some conditions, Preferring and High Alcohol-Drinking rats self-administer enough alcohol to achieve blood alcohol concentration levels of 200 mg% or greater (Murphy et al. 1986), but more generally, Preferring rats appear to drink for the pharmacological effects and will stop self-administration when blood levels reach 50–70 mg% (Waller et al. 1982a). These levels correspond to 0.05 and 0.07%—most US states now outlaw driving at either the 0.05 or 0.08% level. Preferring rats develop metabolic and neuronal tolerance (Lumeng & Li 1986) and dependence (Waller et al. 1982b) with chronic free-choice ethanol drinking.

Another widely used set of selected lines was bred for the severity of withdrawal symptoms when chronic alcohol exposure was discontinued. Alcohol withdrawal convulsions have been reported to occur in all animal species, including humans. Duplicate lines of mice Withdrawal Seizure-Prone or -Resistant to alcohol withdrawal convulsions following a period of chronic alcohol vapor inhalation were developed (Crabbe et al. 1985). These lines did not differ in sensitivity to several effects of ethanol, or in the magnitude of tolerance development, but Withdrawal Seizure-Prone and -Resistant mice differed in withdrawal severity from diazepam, phenobarbital, and other sedative-hypnotic drugs (Belknap et al. 1987, 1988, 1989). Furthermore, Withdrawal Seizure-Resistant mice were found to drink more alcohol than Withdrawal Seizure-Prone mice in a preference test (Kosobud et al. 1988),

consistent with the negative genetic relationship seen in inbred strains, discussed earlier (Metten et al. 1998). They also differed in sensitivity to ethanol place and taste conditioning (Chester et al. 1998). Many other differences between these lines have been reviewed (Crabbe 1996). Although the results of these lines are not obviously consistent with the low response in subjects at risk for alcoholism reported by Schuckit (2000), they suggest that this set of selected lines might offer a model for a genetic propensity to polydrug abuse.

Selection remains a powerful tool in the arsenal of the genetic animal modeler. In the case of drug dependence, many of the contributing factors obvious from a pharmacological perspective have been modeled in selected lines. A particularly useful future strategy might be to breed selectively for and against expression of other traits with presumptive relevance for the intrapsychic effects of drugs, and/or for those personality and behavioral traits found to be comorbid with addiction risk in human populations, such as impulsivity, antisocial behavior, and depression. Highly impulsive mice could then be compared with a low-impulsivity line to see whether they also displayed differences in abusive drug self-administration. Such traits are, of course, intrinsically more difficult to model convincingly in rodents (Altman et al. 1996), but some attempts have been made, particularly for depression (Weiss et al. 1998, Overstreet 1993) and for anxiety, assessed in an elevated plus maze (Liebsch et al. 1998a,b). Rats bred for High-Anxiety-Related Behavior have been found to drink less alcohol than those bred for Low-Anxiety-Related Behavior (Henniger et al., submitted).

QUANTITATIVE TRAIT LOCUS MAPPING   Recent advances in molecular biology have led to an unprecedented explosion in knowledge about the physical aspects of our genes. This has allowed neuroscientists for the first time to begin to translate statistical statements about genetic risk into knowledge of the specific regions on specific chromosomes where genes of importance have been localized. This is a huge first step toward the ultimate identification of those genes and ascertainment of their function. The implications of these technologies for psychology have been discussed in more detail elsewhere (Wahlsten 1999, Plomin & Crabbe 2000).

Studies with genetic animal models in multiple laboratories have established a dense genetic map of distinct DNA sequences scattered throughout mouse and rat chromosomes. Because of our evolutionarily shared ancestor, humans and mice share approximately 80% of these sequence juxtapositions. Practically, this means that when a specific gene's location has been identified in mice, the location of the homologous gene is known in humans 80% or more of the time. During the past 10 years many studies have demonstrated that individual differences in genetic response to drugs of abuse can be reliably associated with particular regions of the genome [termed "quantitative trait loci" (QTLs)]. Whenever the degree of drug sensitivity is reliably associated with a QTL, this implies that a specific allelic form of a specific gene or genes in that region leads to altered drug sensitivity.

There are now more than 30 QTLs mapped for drug response traits in mice, using inbred strains, their F2 and backcross generations, recombinant inbred strains,

selected lines, and congenic strains (Crabbe et al. 1999a). The responses mapped range from drug sensitivity (Deitrich et al. 2000, Gehle & Erwin 2000, Jones et al. 1999), tolerance development (Gehle & Erwin 2000, Deitrich et al. 2000), withdrawal severity (Buck et al. 1997, 1999), reinforcement (Risinger & Cunningham 1998), and tendency toward self-administration. QTL studies are beginning to integrate behavioral and neurobiological analyses. For example, QTLs for the severity of withdrawal from alcohol and pentobarbital have been identified in largely the same regions of mouse chromosomes (Buck et al. 1997, 1999), and one region includes genes that code for several of the subunits of the $GABA_A$ receptor. Further studies show significant association between one variant form of the $GABA_A\gamma 2$ subunit gene and alcohol withdrawal severity across a panel of inbred strains (Hood & Buck 2000). Although this does not prove that the $\gamma 2$ subunit gene is actually responsible for the original QTL association, it is promising that this gene cannot be excluded and supports further efforts to test this candidate gene. It is also encouraging that some human QTL studies with alcohol-dependent subjects have found evidence for an association with this cluster of $GABA_A$ receptor subunit genes (Sander et al. 1999; Loh et al. 1999, 2000; Iwata et al. 2000).

The largest group of studies has mapped genes related to alcohol preference drinking in mice. These studies have been reviewed elsewhere (Crabbe et al. 1999a, Phillips et al. 1998a), but the genetic locations identified have been very similar across laboratories, despite the use of different specific tests of alcohol preference and different mapping populations. Newer studies have also found similar map locations (Vadasz et al. 2000, Whatley et al. 1999). These studies have suggested several candidate genes, some of which have been tested (see next section).

Many studies are now accumulating that do not target addiction-related responses directly but may be of some relevance owing to the comorbidity of anxiety-related disorders and addictions. QTLs for activity in an open field (Gershenfeld et al. 1997; Gershenfeld & Paul 1997, 1998; Turri et al. 1999) and for contextual fear conditioning (Caldarone et al. 1997, Wehner et al. 1997) have been mapped in mice. An interesting, related project in rats has explored the use of factor analyses of data from several behavioral tasks including open field activity to derive factors thought to reflect anxiety. These studies have used multiple rat inbred strains as well as crosses. QTLs for these factors have then been identified. The studies have shown relationships between the serotonin system, stress-related responses, and anxiety-like behavior, reflected as overlapping QTL regions and as co-contributors to specific factors (Castanon et al. 1995; Courvoisier et al. 1996; Kulikov et al. 1995; Moisan et al. 1996; Ramos et al. 1997, 1998, 1999; Ramos & Mormède 1998). Relevance to addictions was addressed in a study that factor analyzed 13 behavioral variables in a number of rat lines selected for high or low alcohol consumption. Although not all variables usually taken to reflect anxiety were correlated across lines, for some variables there was a clear negative association with alcohol preference. That is, high-preferring genotypes loaded lower on factors reflecting anxiety than low-preferring genotypes (Overstreet et al. 1997). And, as noted above, selection for high anxiety was related to high alcohol preference (Henniger et al., submitted).

The current problem is refining these genetic maps to the point that only a few genes are contained in the QTL confidence interval. No group has yet successfully identified a gene through QTL mapping for an addictive drug response, but this has been achieved for some other traits. For example, one QTL for hypertension in rats was subsequently demonstrated to be the gene encoding angiotensin-converting enzyme, known to be important in regulating blood pressure (Jacob et al. 1991). Several groups around the world are using these methods to close in on genes important for addictions. Fehr et al. (submitted) have used specially bred, congenic strains to reduce the confidence interval surrounding a barbiturate withdrawal QTL to a region containing fewer than 20 genes. In parallel, human association and linkage studies are seeking analogous statistical evidence for QTLs related to alcoholism, depression, substance abuse, and other related traits (Foroud et al. 1998), although these studies are intrinsically much more difficult in human populations for a number of technical reasons (see Gelernter 1999).

TARGETED MUTAGENESIS   Sometimes a QTL confidence interval contains a gene whose function appears to be highly relevant to the trait being mapped, as in the example of the $GABA_A$ receptor subunit genes and drug withdrawal given above. Either in pursuit of the genes responsible for QTLs or because a particular gene product is implicated in an addictive behavior based on other neurobiological evidence, investigators have turned to the study of targeted mutants to explore the role of particular proteins. A specific gene can now be inserted into a mouse's germ line, and an over or underexpression transgenic animal studied. Alternatively, the gene can be disrupted or deleted entirely, creating a null mutant or knockout. Many such mutant mice have been shown to display altered drug responses (for review, see Buck et al. 2000). For example, considering only alcohol preference drinking, the genes thus far effectively targeted, or implicated by mapping strategies, include the gene for the serotonin-1B receptor subtype (Crabbe et al. 1996; but see Phillips et al. 1999, Crabbe et al. 1999b), the dopamine D1 and D2 receptor subtypes (El Ghundi et al. 1998, Phillips et al. 1998b), the neuropeptide Y2 receptor gene (Thiele et al. 1998), the protein kinase A gene (Thiele et al. 2000), the protein kinase C epsilon gene (Hodge et al. 1999), the β-endorphin gene (Grisel et al. 1999), and others. In each of these cases, a significant difference was reported in alcohol preference drinking between the null mutant and its control. However, in much the same way that lesioning a brain area and finding a subsequent difference in behavior does not identify that brain area unequivocally as the biological source of the behavior, results from null mutants must be interpreted cautiously. A primary source of caution is the fact that such mutants experience their entire developmental course lacking the deleted gene product, and the highly plastic brain has sought to compensate in unpredictable ways for whatever functions were disrupted (see Wehner & Bowers 1995, Uhl 1999, Gerlai 1996).

RANDOM MUTAGENESIS   Mutations can also be induced at random throughout the genome through X-irradiation of mice (an older technology) or through treatment with a mutagenic chemical such as N-ethyl-N-nitrosourea. A number of large-scale

projects have recently begun in which thousands of mice are mutated and their off-spring screened for behavioral and neurobiological abnormalities (Nolan et al. 1997, 2000; de Angelis et al. 2000). If a phenotypically deviant mouse is then bred, and proves fertile, its offspring should also carry the mutated gene, which can then be mapped and identified using the methods alluded to in the QTL mapping section. These methods are too new for it to be known whether they will be efficacious in identifying relevant mutated genes for complex traits, but they are currently highly touted (Nadeau & Frankel 2000). A more balanced assessment of their prognosis suggests that it may be difficult to apply them to the case of complex traits, in which multiple, small gene effects are the rule (Belknap et al. 2001).

Expression Arrays/Gene Chips

Current technology has provided the genetic research community with the power to ask which genes are more or less active in directing synthesis of their protein products (Watson & Akil 1999). With the recent near-completion of the map containing all human genes by the Human Genome Project (Lander et al. 2001, Venter et al. 2001) have come the current generation of gene chips. Using one of several technologies, snippets representing many thousands of individual gene sequences have been bonded to tiny chips (e.g., glass plates). When a sample of DNA is applied, those genes actively expressed in the sample bind to their embedded ligand, and the resulting interaction is visualized. At least 6000 mouse brain DNA probes are available on chips, and the first studies are beginning to identify genes differentially expressed in brain tissue from alcoholics vs controls (Lewohl et al. 2000) and in adrenal tissue from mice acutely withdrawing from ethanol (Thibault et al. 2000). (For review, see Reilly et al. 2001.)

Another study showed a specific pattern of gene-expression changes in nucleus accumbens tissue from primates exposed to cocaine for over a year, including protein kinases and other cell regulatory genes (Freeman et al. 2001). A recent review summarizes the roles that changes in gene expression are likely to play in the addictive process (Nestler 2000). One goal driving a great deal of the interest in gene expression profiling work is the hope that new genes will be identified that will lead to the development of novel drugs useful in therapy (Hefti 2001). Drugs could also be tailored to maximize an individual's response by using specific knowledge of an individual's genotype.

Use of these techniques will increase exponentially for the next several years, and they bring a new challenge—that of making sense of the data. A typical gene-chip expression array analysis identifies dozens of genes whose expression is increased or decreased as a function of the diagnostic or treatment group compared with controls. Occasionally, expression is drastically altered [e.g., exposure of cells to alcohol chronically led to a 20-fold increase in expression of dopamine beta hydroxylase (Thibault et al. 2000)]. Much more common, though, is the finding of numerous genes whose expression is changed about 100–200%. Numerous statistical problems attend the analysis of these studies, including detecting which

are true gene expression differences and which are false positive changes, detecting changes in genes with intrinsically low expression levels, and foremost, determining what the pattern of changes in expression of all those diverse genes means to the organism's function (i.e., the behavioral genomics issue).

The current state of the art for such analyses classifies genes according to broadly (and ill-defined) functions such as "cellular metabolism" or "cellular signaling." The categories reveal the intrinsic orientation toward a reductionist and proteomics perspective: They are oriented toward explaining the functions of the protein in the immediate environment in which it is synthesized and acts. A behavioral genomics perspective will be useful here, where the pattern of gene expression is related not only to cellular function per se but also to the behavioral functions in the whole organism that attended the original treatment or diagnostic comparison.

GENES AND THEIR ENVIRONMENTS

The existing genetic animal models have taught us a great deal about the basic pharmacology, physiology, and biochemistry of drugs' effects on the nervous system. They have unequivocally proven that a substantial proportion of individual differences in response to or avidity for drugs of abuse is genetically influenced. Recent studies have begun to isolate the genes responsible for such individual differences. In addition to (and in some cases building on) these gene mapping efforts, candidate gene approaches have also implicated many specific genes as important for drug responses. Three sources of complexity beyond that introduced by pleiotropy need to be considered.

Gene-Environment Interaction

By definition, the behavior of individuals with particular genotypes can only be assessed in an environment, and systematic changes in the environment clearly affect the behavioral outcome. Abundant evidence reveals that different genotypes respond differentially to environmental manipulation. This is termed "gene-environment interaction," and is crucial to the reasonable interpretation of the range and limits of genetic influences on behavior. Ninety-eight recombinant inbred *Drosophila* strains were reared in three environmental conditions, standard medium at two temperatures, and one medium-temperature combination with ethanol added. QTL effects on fitness (reproductive success) could be estimated from the strain means, and the effects of the QTLs depended on the rearing medium (Fry et al. 1998). In another example, inbred strains of mice showed differing degrees of willingness to drink an offered alcohol solution (McClearn & Rodgers 1959). When increasing concentrations of alcohol were offered, some strains (e.g., C57BL/6) continued to self-administer the drug to water, whereas others (e.g., A/HeJ) began to reject it at higher concentrations (Rodgers & McClearn 1962, Belknap et al. 1993). Genotypic differences in alcohol self-administration were also shown to differ in different cage types and according to how food was

presented to the rat strains (Adams et al. 2000). Animal-model research in addictions has provided many such examples.

The utility of animal models for exploring gene-environment interaction is especially high because both genotype and environment can be manipulated experimentally. However, even under this very high level of control the specific environmental variables that are potent in differentially affecting genotypes can be hard to identify. A recent study asked whether inbred strain differences in behavior would be the same in three different laboratories when as many environmental and genetic variables as possible were rigorously equated (Crabbe et al. 1999b). Mice from several inbred strains and one null mutant were tested at exactly the same ages on exactly the same days on a battery of six behaviors. The animal husbandry was nearly identical (same laboratory chow, cages changed on the same days, etc.). The apparatus (e.g., elevated plus mazes, water mazes) and test protocols (including how animals were handled) were nearly identical. However, there were some variables that it was impractical to standardize completely, such as the local water and air in the animal facility, and the individual experimenters in the three locations.

As expected, the strains differed a great deal in all behaviors, and for some tests (such as alcohol preference drinking) there were no strong indications of differences in the strain pattern of alcohol preference across sites. However, for some tests (such as the tendency of mice to venture onto the open arms of an elevated plus maze, generally taken as an index of anxiety), there were significant strain X laboratory interactions. In general, the weaker the overall genetic influence on a trait (i.e., the lower the heritability), the more likely there was to be a genotype X–environment interaction.

Do the results of this study imply that behavioral tests in laboratory mice are intrinsically unreliable? Reliability was generally high at each site. The alternative interpretation is that even differences in environmental test situations that are not obvious to the experimenter may have great importance for the animal and may affect animals with different genotypes differently (Crabbe et al. 1999b).

One implication of this finding is that the particular behavioral test employed to assay a given behavioral domain may affect the interpretation of the results. This is potentially a large problem, particularly for experiments characterizing null mutants. For example, an early study with a null mutant lacking one of the multiple variants (5-HT_{1B}) of receptors for the neurotransmitter serotonin found that the knockouts were much less susceptible to the intoxicating effects of an acute alcohol injection than were the wild-type control mice in a test called the grid test (Crabbe et al. 1996). However, a subsequent study tested these animals for alcohol's incoordinating effects using several other tasks, including frequently used tasks such as the rotarod. Null mutants were found to be less sensitive to alcohol on some, but not all, of these tasks. This implies that these different behaviors must not all represent a single, monolithic domain, and a careless investigator might conclude that the 5-HT_{1B} receptor gene was important for "alcohol-induced ataxia," whereas a more careful analysis would reveal that it affects some but not

all of the contributing behaviors (e.g., balance, intact proprioceptive feedback, patterned gait, muscle strength, etc.) (Boehm et al. 2000).

Although more difficult to demonstrate in humans, in whom the genotype is much more difficult to control rigorously, gene-environment interaction clearly exists. For example, a classic study of the genetic susceptibility to alcoholism and related behaviors compared Scandinavian men at (or not at) genetic risk (based on diagnosis of close relatives) for one of two broadly defined variants of alcoholism. Type I alcoholism is characterized by relatively mild abuse, minimal criminality, and passive-dependent personality variables, whereas Type II alcoholism is characterized by early onset, violence, and criminality, and is largely limited to males (Cloninger 1987). Multiple variables in the rearing environments were assessed, and individuals were subsequently classified as having been raised in either risk-promoting or protective environments. Individuals at genetic risk for Type I alcoholism were more often diagnosed, demonstrating genetic influence, but this tendency was much more pronounced when they also had higher-risk environments. For Type II alcoholism, genetic loading also increased diagnoses, but there was little further elevation if the rearing environment was also risky (Cloninger et al. 1981). This outcome illustrates the concept that the same environmental risk factors can play a very different role depending on an individual's genotype.

On at least three counts, this is a great oversimplification of a very complex analysis. First, whereas many different diagnostic typologies have been proposed for alcoholism, nearly all support the existence of at least two broadly differentiable variants of the disease (Johnson et al. 1996a, Litt et al. 1992, Babor et al. 1992). Second, multivariate statistical methods were used to categorize variables in the subjects' environmental background as risk-promoting or protective. Finally, a similar analysis of a sample of female alcoholics provided a somewhat different outcome (Bohman et al. 1981). Nonetheless, the interaction seen was substantial.

Gene-Environment Correlation

Genotypes are often not randomly represented in environments. A frequent contributor to relapse to substance abuse is thought to be succumbing to the environmental triggers represented by myriad cues in the patient's environment, e.g., seeing the house where he habitually purchased drugs, hanging around with other drug users. Studies have demonstrated the potency of exposure to previously drug-related cues in eliciting both craving for drugs and increases in physiological responses such as heart rate and pupil diameter (Childress et al. 1999, O'Brien et al. 1998). At least some contribution to substance abuse is likely to be the tendency of genetically susceptible individuals to remain in the risk-promoting environment, thereby potentiating their overall risk. Indeed, many therapies strongly advocate making radical changes in the day-to-day living situation of recovering addicts, a prescription that is unfortunately difficult for many to follow due to limited socioeconomic choices (Budney & Higgins 1998).

It is obviously difficult to conduct controlled studies of gene-environment cor- relation with humans. However, one underlying principle is that drug responses can often depend upon conditioning to environmental cues. Many animal studies have documented genetic differences in sensitivity to drug-conditioned responses, such as place and taste conditioning (Broadbent et al. 1996, Cunningham 1995, Cunningham et al. 2000, Risinger & Cunningham 1998). The role of conditioning in drug abuse has been reviewed elsewhere (Altman et al. 1996, O'Brien et al. 1998, Robbins & Everitt 1999).

Epistasis

Whenever more than one gene affects a trait, epistatic interactions may be at work. Complex behaviors, including those contributing to addictions, are influenced by many genes, each with relatively small independent effect. Epistasis is the statistical interaction of such individual gene effects. The simplest case is that in which the presence or absence of a particular allele at a second gene significantly modulates the effect of allelic differences at a gene of interest (Browman & Crabbe 1999). A recent example is not strictly about addictive behavior, but rather anxiety, which is extensively comorbid with the addictions. Three groups interested in the stress axis recently independently produced mice in which the corticotropin-releasing hormone (CRH) receptor-2 gene (*Crhr2*) had been deleted. Because of the well- established role of CRH in modulating anxiety-like responses (Weninger et al. 1999, Skutella et al. 1994), all three groups used the elevated plus-maze anxiety test as well as the classic open field test, and each tested both sexes. Many additional variables were also assessed in each study. Although the findings were internally consistent within each group's results, they differed markedly in their conclusions about the role of the CRH-R2 receptor gene in anxiety. One group (Coste et al. 2000) saw no effects on anxiety-related behavior in either sex, whereas the second group (Bale et al. 2000) found greater anxiety in both male and female knockouts. The third group (Kishimoto et al. 2000) saw greater anxiety in male knockouts only, and in only one test.

The source of these differences could simply be that different apparatus, light- ing, handling conditions, and other test procedures were used, idiosyncratic to each group. We have already seen that even when such variables are carefully standard- ized, genetic differences play out differently in different environments. Another possibility is that the CRH-R2 receptor gene has no consistent role in modulating anxiety, but each group used multiple, putative tests of anxiety and each obtained largely consistent results across tests.

It seems more likely that epistasis was at work. The genetically engineered constructs inserted into the embryonic stem cells to produce the gene deletion were different in each laboratory, and each carried a relatively long piece of DNA along with the targeted gene. Thus, other closely linked genes could have been introduced to the recipient mice, and these necessarily differed from laboratory to laboratory. These "passenger genes" could have been interacting epistatically

with the CRH-R2 receptor gene to influence behavioral outcomes, such that a given anxiety response was only expressed in the presence of a specific, additional gene in addition to the loss-of-function variant of the CRH-R2 receptor gene. Furthermore, each group introduced its null mutant into a different substrain of 129 inbred mice (these 129 mice served as the source for the embryonic stem cells into which the null mutation was introduced). Different substrains of 129 are very similar genetically, but not identical (Simpson et al. 1997). The mice tested also had varying percentages of the C57BL/6 inbred strain genome, so the effects of the targeted gene could also have been interacting with genes differing in the genetic background, as well as with passenger genes in the construct (Gerlai 1996). There are other possible contributors to these behavioral differences, discussed more fully in the three papers mentioned above and elsewhere (Crabbe 2001).

Researchers are beginning to look for these sorts of interactions in their gene mapping efforts, and it is not surprising that epistasis appears to occur frequently. In a QTL analysis from our group, we were able to demonstrate a significant difference in acute pentobarbital withdrawal severity between mice homozygous for DBA/2J strain alleles in a region of chromosome 11 and those homozygous for C57BL/6J alleles (Buck et al. 1999). This indicated the presence of a gene in this chromosomal region where the DBA/2J-specified gene tended to reduce withdrawal as compared with the C57BL/6J gene. A recent analysis of epistatic interactions, however, showed that this was only true when the animals had DBA/2J alleles in a second region, on the distal end of chromosome 1. If mice had C57BL/6J alleles on distal chromosome 1, there was no difference in withdrawal between mice with C57BL/6J and DBA/2J genomes on chromosome 11 (Hood et al. 2001).

Genes can obviously interact in multiples greater than two. The field is just now beginning to study such interactions, and extension of such analyses to multigenic interactions will require a daunting degree of statistical power. This is because power to detect interactions requires much greater numbers of subjects than are required to detect main effects (Wahlsten 1990). Nonetheless, understanding the complexity of genetic interactions will be crucial. After all, the relevant clinical traits are extensively comorbid (e.g., alcoholism, other substance abuse, impulsivity, attention deficit/hyperactivity disorder, depression, etc.), and their comorbidity is likely to represent a mixture of genetic and nongenetic sources (Crabbe 1999).

TAKE HOME MESSAGES

Interpretation of Genetic Differences

The ubiquitous influence of genes on addiction-related traits must not be over-simplified. "The gene for . . ." syndrome understandably infects the popular press (although scientists have a clear responsibility to lobby strenuously against this kind of reporting). Unfortunately, for many molecularly oriented neuroscientists, a limited result identifying a specific gene with a specific behavioral outcome often also leads to over-naive interpretation. For complex traits, the general rule seems to

be that the aggregate contribution of the many genes contributing toward individual differences is no more than 50% of the variance—the rest is explicitly not genetic. Whereas some would argue that teasing apart the relative contributions of genes and environments is a near impossibility (Gottlieb 1998), the general view is that specific domains of genetic influence can, with care, be identified. Nonetheless, it is important to remember that many genes contribute to complex traits, interacting with each other as well as the environments in which they are expressed.

What Specific Contributions Can Animal Genetics Make to the Addictions?

One suggestion raised earlier in this review was that insufficient use has been made of perhaps the most powerful of all behavior genetics techniques, artificial selection. If one wishes to deconstruct the contributions of disinhibition to drug-seeking behavior, it would be a straightforward matter to breed mice for high or low "novelty-seeking" responses and then see whether they differed in drug self-administration. It has been shown that when rats are exposed to an open field, a situation whose novel features cause mild stress, they display different levels of locomotion. High responders can then be shown subsequently to self-administer psychostimulant drugs to a greater degree than low responders. Reviews of these and related studies discuss the neuroendocrine and behavioral profiles characterizing these groups (Piazza & Le Moal 1996, Bardo et al. 1996).

From a genetic perspective, these studies offer no evidence that the behavioral differences are genetic as opposed to environmental. However, simply attempting to select for the novelty response would rapidly answer the question. Of course, the studies reviewed above suggest that it would not necessarily be easy to do this experiment. For example, breeding for high vs low scores on a signaled nosepoke task (Logue et al. 1998) might, or might not, lead to parallel divergence in scores in a delay or probability discounting task (Richards et al. 1997) or in a delayed reinforcement of low rate operant task, even though all three tasks are thought to assay impulsivity. Still, such experiments would be a worthwhile undertaking and could offer much useful insight to the predisposition/comorbidity issues surrounding human genetic studies.

A second area of contribution is the identification of specific genes for risk for or protection from addictive behavior (see "Quantitative Trait Locus Mapping"). The rapid pace of technological development in genetic markers [e.g., the development of many thousands of new genetic markers, much more densely spaced, by ascertaining single nucleotide polymorphisms (Lindblad-Toh et al. 2000, Cargill et al. 1999)] will make the path from QTL to responsible gene much easier in the near future.

Will the proliferation of genetic studies in animals resolve all the most vexing issues facing addiction studies? Almost certainly not. Even this brief review has made it clear that any single addiction diagnosis is etiologically heterogeneous, which probably means that it is both genetically and environmentally heterogeneous

as well (not to mention heterogeneous at the intersection of genotype and environment, and so on). Certainly, the history of genetic animal-model research suggests that such studies have great potential for furthering our knowledge of how drugs work in the brain. There is hope among behavioral geneticists that use of genetic information could lead to better diagnostic approaches (Plomin & Crabbe 2000), but the principal, and probably insurmountable, problem is the small effect size of most specific genes of importance. And there is a strong probability that behavioral genomics studies in animals will lead to better therapeutic agents than those currently available. It may not be necessary to identify all the influential genes to devise novel strategies for prevention and treatment of complex disease traits.

Addiction in the Postgenomics World

The rapid proliferation of genetic data–gathering capability has found many a scientist in possession of the DNA from many patients/subjects. Because it is now so comparatively sraightforward to genotype those samples, the potential for misuse of genetic information is great. A fraction of the resources of the Human Genome Project has been devoted to the study of the Ethical, Legal and Social Implications (ELSI) Program and others with similar goals (see http://www.lbl.gov/Education/ELSI/ELSI.html). Privacy and confidentiality issues and their implications for employment and insurance are an extremely complex area. In addition, patients whose DNA leads to patentable discoveries are beginning to sue for a share of the profits.

The need for serious ethical discussions is clear, but the answers will not be simple. As has been the central message of this review, at the heart of the problem is the small effect size of any individual gene contributing to complex traits. The ethical issues surrounding a diagnosis of Huntington's disease are difficult enough, and this is a single-gene disorder for which a yes/no answer to genetic risk can be given. What does it mean to know that an individual has 3 of the 15 (or is it 30?) "bad" genes, e.g., those predisposing to alcoholism, versus having 8 of the 15? Obviously, everyone would prefer the former diagnosis to the latter, but absent knowledge of whether alcoholism is a threshold character or a continuous trait and how the various risk-promoting genes interact with each other in specific combinations, and without environment-related information that is as sophisticated and articulated as the genetic information on risk, it is difficult to see what the appropriate ethical choices are. This does not mean that regulation will not be attempted before the science is clear. Several states have introduced laws regulating genetic privacy.

I am not a bioethicist, but I have been repeatedly exposed to ethical issues during a career devoted to chasing genetic sources of influence on complex traits. In my perusals of the literature relating to ethical decision-making vis à vis genetics, I have been struck by the persistent tendency to raise, rather than answer, questions such as those raised above. The reader is directed to a recent review for other sources of relevance to the ethical questions, where we perpetuated this tendency (Crabbe & Belknap 1998). In addition, the National Institutes of

Health maintains several links to web sites of relevance to ethnicity and genetics, gene patenting, genetic testing/counseling, and gene therapy/gene transfer (http://www.nih.gov/sigs/bioethics/). I suspect that no one with more than a little knowledge in the related scientific areas feels capable of prescribing ethical guidelines. Nearly all scientists I know would agree that it seems unfair, immoral, or a violation of privacy for an insurance company to obtain access to a patient's DNA information without explicit permission and decide that a high risk for a disease justified a higher premium. However, they are much more divided on the issue of whether a patient should retain privacy rights blocking the use of his or her DNA information, freely given with informed consent for a particular genetic linkage study, in a future genetic linkage study for a different trait, in which personal patient-identifying information is doubly blinded. Entire books have been devoted to these complex issues, and it is simply beyond the scope of this review to pursue them in any reasonable depth. The interested reader may also find useful the text of a February 2000 ELSI Research Planning and Evaluation Group Report covering the first 10 years of the ELSI Programs and future plans, and the links cited therein (http://www.nhgri.nih.gov/ELSI/erpg_report.html).

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