Genetic Animal Models of Alcohol and Drug Abuse

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Behavioral and pharmacological responses of selectively bred and inbred rodent lines have been analyzed to elucidate many features of drug sensitivity and the adverse effects of drugs, the underlying mechanisms of drug tolerance and dependence, and the motivational states underlying drug reward and aversion. Genetic mapping of quantitative trait loci (QTLs) has been used to identify provisional chromosomal locations of genes influencing such pharmacological responses. Recent advances in transgenic technology, representational difference analysis, and other molecular methods now make feasible the positional cloning of QTLs that influence sensitivity to drugs of abuse. This marks a new period of synthesis in pharmacogenetic research, in which networks of drug-related behaviors, their underlying pharmacological, physiological, and biochemical mechanisms, and particular genomic regions of interest are being identified.

Behavior is determined jointly by genetic and environmental influences, but the complexity of behavioral phenotypes usually makes straightforward analysis of genetic influences difficult. The development of genetic animal models has facilitated studies of the genetic basis of drug-related behaviors and made pharmacogenetic research an exception that may surmount this difficulty. "Alcoholism" and "addiction" are typically conceptualized as comprising multiple, interacting behavioral components, some influenced by genetic factors and others by nongenetic, or environmental, factors (*1*). Accumulating evidence suggests that there is a genetic predisposition to abuse drugs. For human studies, this is most clear for behaviors indicative of alcohol abuse in males, such as frequency or intensity of drinking (*1, 2*) and for smoking (*3*). The tendency to abuse alcohol seems to be codetermined with the tendency to abuse other drugs (*4*). Furthermore, psychosocial variables clearly affect the propensity for drug abuse, and predisposing characteristics such as antisocial personality are also influenced by heredity (*5*). However, the specific genes that code for proteins that increase or decrease susceptibility to drug abuse remain largely unknown.

Genetic Animal Models

Most pharmacogenetic research has used genetic animal models to understand how drugs exert their addictive effects and to predict individual susceptibility to those effects (*6*). Genetic animal models have several advantages (*7*), including (i) the experimenter is in control of the subject's genotype; (ii) numerous stable genotypes of rat and mouse are available, which allows cumulation of knowledge; and (iii) many forward and reverse genetic techniques are applicable (*8*). They also have one principal limitation: genetic animal models are only simulacra of their more complex human conditions (*9*). Thus, we may not find or develop a truly alcoholic rat or a cocaine-addicted mouse, but individual features of the complex of drug-related behaviors and neurobiological responses can be modeled and studied successfully. Orderly dose-effect relations typically govern the magnitude of drug-induced behavior, and the discipline of pharmacology provides the necessary tools to examine the potential role of many specific targets, such as synthetic and catabolic enzymes, neurotransmitter receptors and transporters, ion channels, and second messengers.

Genetic animal models have been most thoroughly and successfully applied in the investigation of (i) sensitivity to initial or adverse drug effects; (ii) neuroadaptation underlying chronic tolerance or sensitization, dependence, and withdrawal; and (iii) drug reward (or aversion), which is related to the pleasurableness of drug administration in humans. Any or all of these factors may determine genetic risk. For example, in genetically susceptible young men, low initial alcohol sensitivity may predict susceptibility to the development of alcoholism many years later (*10*). The identification of phenotypic or genetic markers that can predict drug abuse susceptibility directly and noninvasively could prove of great clinical and diagnostic importance (*11*).

Progress in genetic studies of drug responses has been achieved with two approaches first used in the 1950s—selective breeding and analysis of standard inbred strains. Mardones (in Chile) and Ericksson (in Finland) separately developed, by selective breeding, rat lines that differ markedly in the amount of ethanol they will drink voluntarily (*12*). Selected lines differ genetically principally in those genes affecting the selected-for trait: therefore, other differences between them suggest that the selected genes also have other effects. Selective breeding has been most widely used to select for ethanol-related traits but has also been used for opioids, benzodiazepines, and other psychoactive drugs, and its success demonstrates the existence of genetic influence on these traits. Table 1 summarizes the currently available lines selectively bred for traits that may be related to drug abuse.

In 1959, McClearn noted differences among several standard inbred strains of mice in their preferential drinking of alcohol (*13*). When members of an inbred strain (which by definition are virtually genetically identical) are tested in controlled environmental conditions, differences among strains reflect genetic determination; strain similarities on multiple traits suggest common genetic influences.

Sensitivity Models

Genetic influences appear to be nearly universally important in determining sensitivity to drugs. Several hundred reports have appeared documenting genetic differences in sensitivity or toxic response to almost all drugs subject to abuse (*12, 14*). However, very few of these differences have been adequately explained in terms of the molecular mechanisms or identification and mapping of any specific genes responsible. Here, we wish to focus on three examples of what can be accomplished in this burgeoning research area.

Long-Sleep (LS) and Short-Sleep (SS) mice are a widely used genetic model of drug sensitivity that were selected for differential duration of righting reflex suppression by ethanol ("sleep time"), an index of anesthesia. As a result, these two lines differ markedly in central nervous system (CNS) sensitivity to ethanol (*7, 15*). They also differ in sensitivity to a number of benzodiazepines, barbiturates, and gaseous anesthetics, which indicates the inherited influence of common genes and suggests that a genetic difference in neural transmission at γ-aminobutyric acid (GABA) synapses (a target of both benzodiazepines and

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barbiturates) may be important. Exposure to ethanol enhances $GABA_A$ receptor–mediated ^{36}Cl uptake by brain membrane vesicles from LS but not SS mice (*16*). The activity of GABA-activated chloride channels in *Xenopus* oocytes expressing LS brain messenger RNA (mRNA) are also enhanced by ethanol, whereas the currents in oocytes expressing SS mRNA are inhibited (*17*). Co-injection of antisense mRNA for the γ_{2L} subunit variant of the $GABA_A$ receptor indicates that expression of this subunit is required for ethanol sensitivity of LS $GABA_A$ channels (*18*). A genetic difference in one or more of the protein subunits of the $GABA_A$ complex is also suggested by differential sensitivity between LS and SS mice to heat inactivation of receptor binding (*19*). Alternative splicing of the γ_2 subunit to produce γ_{2L} (long) causes insertion of eight amino acids, including a consensus sequence for phosphorylation by protein kinase C (PKC). Site-directed mutagenesis of this domain demonstrated that it is required for potentiation of $GABA_A$ receptors by physiological concentrations of ethanol (*20*). Furthermore, PKCγ "knockout" mice display reduced sensitivity to the effects of ethanol on loss of righting reflex and body temperature, but they have normal responses to flunitrazepam and pentobarbital (*21, 22*). Likewise, $GABA_A$ receptor function in isolated brain membranes showed that the PKCγ null mutation abolished the action of ethanol, but not flunitrazepam or pentobarbital (*21*). These studies show the interactions of ethanol with $GABA_A$ receptors and suggest that PKC isoenzymes may be important as determinants of genetic differences in response to ethanol.

A second line of research began with the development of seven recombinant inbred (RI) mouse strains from a cross of the progenitor C57BL/6By and BALB/cBy inbred strains (*23*). One of these, the CXBK strain, is deficient in brain μ- but not in δ-opioid receptors compared with other CXB RI strains (*24*) and may be used as a naturally occurring "knockdown" mouse to identify behaviors possibly influenced by μ-opioid receptors (they would be reduced in CXBK mice). Interestingly, the CXBK strain is relatively insensitive to the analgesic effects of morphine, acupuncture, and several stressors, as well as to morphine-induced locomotor activity and respiratory depression (*25, 26*), but is sensitive to the lethal or rewarding effects of morphine or morphine-like opioids (*25, 27, 28*), suggesting that the latter responses are not mediated by μ-opioid receptors and that rewarding effects may be distinct from other responses to opiates.

A third example is drawn from studies of nicotine. In the CNS, nicotine recognizes high-affinity receptors labeled by [^{3}H]nicotine and low-affinity receptors labeled by [^{3}H]bungarotoxin (*3*). Nineteen standard inbred mouse strains show marked genetic differences in behavioral sensitivity to injected nicotine. The density of [^{3}H]nicotine binding sites in brain homogenates predicted sensitivity to the locomotor activity and body temperature effects of nicotine but not its seizure-inducing effects. In contrast, [^{3}H]bungarotoxin binding predicted sensitivity to nicotine-induced seizures but not to the other effects (*3, 29*). Thus, specific genetic differences in receptor number contribute to the marked genetic differences in sensitivity to several effects of nicotine.

Neuroadaptation Models

Withdrawal Seizure-Prone (WSP) and Withdrawal Seizure-Resistant (WSR) mice have been selectively bred for a more than 10-fold difference in the severity of their reaction to ethanol withdrawal (*30*). Although WSP and WSR mice were selected strictly for differences in alcohol withdrawal severity, WSP mice also show more severe withdrawal than WSR mice after chronic intoxication with diazepam, phenobarbital, and nitrous oxide (*31*). WSP mice are so sensitive that they show withdrawal symptoms after a single injection of these drugs, whereas WSR mice do not (*32*). Together, these results suggest coordinate genetic control of withdrawal from several classes of central depressants. In humans, a similar situation might contribute to the tendency of susceptible individuals to abuse multiple drugs (*4*).

There are several neurochemical and behavioral differences between the WSP and WSR lines (*33*). After chronic ethanol treatment, WSP mice exhibit a large increase in the number of brain dihydropyridine-sensitive calcium channels, whereas WSR mice show a much smaller increase (*34*). This difference may partly underlie the distinct difference between the selected lines in neural excitability seen during drug withdrawal. Also, dorsal hippocampal mossy fibers from untreated WSP mice contain 70% less zinc than those from WSR mice (*35*). When they are ethanol-dependent, WSP mice become more sensitive to benzodiazepine receptor inverse agonists. Furthermore, chronic feeding of ethanol to WSP and WSR mice produced changes in mRNA amounts of specific $GABA_A$ receptor subunits that may contribute to withdrawal hyperexcitability, because GABA is the principal inhibitory neurotransmitter (*36*).

Other studies with WSP and WSR mice illustrate the striking genetic independence of withdrawal severity and many other responses to ethanol. WSP and WSR mice do not differ in sensitivity to most other ethanol effects (*37*), supporting the idea that independent genetic factors control ethanol sensitivity, tolerance, dependence, and self-administration and that these traits,

Table 1. Rodent lines selected for drug abuse–related traits. First-listed line in each category shows higher sensitivity on the selected response. References to and sources of these and related lines are available on request. Some of these lines are also maintained as inbred strains.

Line designation	Abbreviation
Initial sensitivity to alcohol	
Long-/Short-Sleep mice [loss of righting reflex (LORR)]	LS and SS
High/Low Alcohol–Sensitive rats (LORR)	HAS1, -2, and LAS1, -2
Alcohol ataxia Nontolerant/Tolerant rats	ANT and AT
COLD/HOT mice (hypothermia)	COLD1, -2, and HOT1, -2
FAST/SLOW mice (open-field activation)	FAST1, -2, and SLOW1, -2
Initial sensitivity to other agents	
High/Low Analgesic Response mice (levorphanol)	HAR and LAR
High/Low cold-water stress-induced Analgesia mice	HA and LA
Diazepam-Sensitive/-Resistant mice (ataxia)	DS and DR
Low/High Diazepam Performance mice (ataxia)	DLP1, -2, and DHP1, -2
Nicotine-Activated/-Depressed mice (open-field activation)	NA1, -2, and ND1, -2
Neuroleptic Responders/Nonresponders mice (haloperidol catalepsy)	NR1, -2, and NNR1, -2
Cocaine Activity High/Low mice (open-field activity)	CAHI1, -2, and CALO1, -2
Pentobarbital Long-/Short-Sleep Time mice (LORR)	LST and SST
Tolerance to and dependence on alcohol	
Withdrawal Seizure-Prone/-Resistant mice	WSP1, -2, and WSR1, -2
High/Low ethanol Withdrawal mice	HW1, -2, and LW1, -2
High/Low Acute Functional Tolerance mice (ataxia)	HAFT1, -2, and LAFT1, -2
Preference for 10% alcohol versus water	
Preferring/Nonpreferring rats	P/NP
High/Low Alcohol–Drinking rats	HAD1, -2, and LAD1, -2
ALKO Alcohol/Nonalcohol rats	AA and ANA
High/low ethanol–consuming rats	UChB and UChA
Sardinian-Preferring/-Nonpreferring rats	sP and sNP
High/Low Alcohol–Preferring mice	HAP1, -2, and LAP1, -2

therefore, have somewhat distinct neurobiological mechanisms.

Studies of neuroadaptive responses to drugs other than ethanol have largely been limited to inbred strain comparisons. Rat and mouse strains differ in diazepam, pentobarbital, phenobarbital, and opiate withdrawal severity (*38*). In 15 standard inbred mouse strains, there was substantial genetic commonality in determination of acute withdrawal severity from pentobarbital, diazepam, and ethanol (*39*).

During chronic drug administration, rat and mouse strains differ in the degree to which they eventually display tolerance (diminution of drug effects) to many effects of drugs of abuse (*14*). However, chronic administration is not always accompanied by tolerance. Particularly for the psychostimulants, such as cocaine or amphetamine (but also for low doses of morphine or ethanol), repeated administration of drugs can lead to sensitization (enhanced responding), which may model the rewarding effects of drugs (*40*). The pattern of strain differences in sensitized locomotor responses to cocaine, opiates, ethanol, and methamphetamine suggests that initial stimulant and sensitized responses to these drugs are probably mediated by at least partially divergent neural mechanisms (*41–43*).

Reward Models

In a classic study with naïve human subjects, an injection of heroin was perceived as pleasurable by some and aversive by others (*44*). A central question in drug abuse research is what causes individual differences: The answer will likely provide important insight into why some individuals abuse drugs and others do not. Clearly, environmental factors are important, but increasingly, genetic contributions are also being recognized as crucial.

The self-administration model most frequently used to detect genetic differences is preference drinking in which the animal is free to drink from two bottles, one containing a drug and the other water. A large degree of genetic influence on voluntary drug consumption has been demonstrated among inbred strains of mice and rats for most drugs abused by people (*13, 45–48*).

Operant self-administration studies require the animal to emit a response (operant) such as a bar press in order for the drug to be administered. Increased operant responding suggests that the drug is rewarding (reinforcement), whereas cessation of responding implies drug aversion. Reports of such studies with inbred strains of rats or mice indicate large strain differences for ethanol, cocaine, and opioids (*25, 46, 49*). The Lewis strain of rat shows much higher rates of operant responding than does the Fischer 344 strain for several drugs of abuse, suggesting that the genetic differences are not drug-specific.

Alcohol Preferring (P) and Nonpreferring (NP) strains of rats were selectively bred for voluntary alcohol drinking under two-bottle choice conditions. P rats also show higher operant responding for ethanol than do the NP rats, indicating a genetically mediated difference in the reward value of alcohol (*33, 50*). Research with these selected lines supports the potential role of lowered nucleus accumbens concentrations of serotonin and dopamine in controlling ethanol drinking (*51*). These differences have been replicated in the High and Low Alcohol Drinking (HAD and LAD) rat lines, also selected for preference differences, and in a segregating population of F_2 rats derived from the cross of P and NP (*52*). The existence of concurrently replicated selection lines (P and NP and both pairs of HAD and LAD lines; see also other available selected lines in Table 1) obviates one limitation of those correlative genetic studies that only compare two strains. It is necessary to study multiple genotypes (strains, selected lines) to conclude definitively that traits are genetically related (*53*).

Another index of drug reward, conditioned place preference, relies on repeated injections of either a drug or saline, each paired with distinct environmental cues. The animals are then tested to determine whether they seek out the cues previously associated with the drug or those paired with saline. Inbred mouse strains demonstrate significant genetic control of conditioned place preference for alcohol and morphine (*28, 54*). Taste conditioning can also be used as an index of a drug's presumed reinforcing effects. Selected line differences in sensitivity to ethanol-induced taste aversion have been reported (*55*).

Compared with studies of neuroadaptation or sensitivity, studies of the genetic determinants of drug reward are in their infancy. In particular, studies of the mediating mechanisms and the mapping of the genes involved have barely begun. Drug reward is a particularly important area in research with genetic animal models. As in human populations, large individual differences in drug self-administration are evident. Furthermore, some laboratory rodents will self-administer virtually all of the same drugs that are abused by humans.

Future Directions

The study of genetic influences on behavior has typically attempted to trace a path from a given behavior to a particular gene. With classical behavioral, genetic, pharmacological, physiological, and biochemical methods and genetic animal models, we are beginning to understand some drug response mechanisms. Yet, questions fundamental to understanding the addictions remain. For example, are genetically influenced addictive behaviors in humans related in any predictable way to normal behavior? Are the biological substrates of addiction to different drugs genetically related? Questions like these are difficult for the current genetic animal models to answer for two reasons: They focus on discrete drug-related traits and on simple genetic structure, such as the pursuit of a single candidate gene. In the terms suggested by Plomin *et al.* (*2*), the research objectives of many of the current genetic animal models have tended to be framed by "one-gene, one-disorder" thinking. However, as pharmacogenetic research begins to deal with the complexity of drug-related behavioral phenotypes more effectively, molecular biological methods are also beginning to make rapid contributions to this area. In the second part of this article, we discuss some research in progress that is facilitating this emerging synthesis of behavioral and molecular genetics. In part, this also reflects the concurrent use of forward and reverse genetic strategies (*8*).

Multivariate Analyses

One method of identifying unifying patterns of genetic regulation of drug responses is to study several standardized genotypes (for example, inbred strains) under controlled environmental conditions. The genetic stability of inbred strains over years and laboratories has allowed the field to accumulate much relevant information for several commonly used strains. In ongoing studies, we and our collaborators have characterized the responses of 15 strains of mice to several doses of four drugs of abuse—ethanol, pentobarbital, diazepam, and morphine. To the extent possible, we have analyzed the same behaviors for each drug (body temperature regulation, open-field activity, and preferential drinking of drug-admixed solutions). Correlations of strain means were then calculated to estimate genetic similarity among responses. For example, the effects of 2 g of ethanol per kilogram of body weight and of pentobarbital at 30 mg/kg to stimulate activity were highly genetically correlated ($r = 0.77$, $P < 0.001$) and appear close together on Fig. 1, thus suggesting common genetic influences. Multivariate statistical approaches allow simultaneous comparison of genetic correlations among several variables. The results of an overall analysis of the genetic similarities among strain responses in two responses, locomotor activity and hypothermia, are shown in Fig. 1.

There is a substantial degree of common

genetic influence of different doses of the same drug on a given response, as expected. Both thermal and activity responses to morphine are genetically related at all doses, and this cluster is distinguishable from the other drugs. Furthermore, responses on both variables to most doses of diazepam and pentobarbital, and the activating effects of moderate doses of ethanol, form a separate cluster, representing a node of common genetic influence. Because all three of these drugs influence $GABA_A$ receptor–gated chloride channels, this is a likely common mechanism that could mediate the genetic similarity of this response cluster. However, the multivariate analyses demonstrate that, unlike pentobarbital and diazepam, the effects of ethanol on body temperature were genetically distinct from its effects on activity. Because genotypes (strains) remain constant, additional commonalities of genetic influence will emerge from such approaches as more traits are studied.

Quantitative Trait Loci Mapping

Quantitative traits are continuously and often normally distributed as a result of polygenic determination at several loci, each a quantitative trait locus (QTL). Mapping these is a formidable challenge because the effects of each QTL may account for only a small portion of the trait variance and must be detected against substantial background variation due to effects of other loci and the environment, and their interactions. Though an individual QTL may have a relatively small effect, in the aggregate they often exert a large influence. This is in contrast to qualitative or discretely distributed traits determined by single locus inheritance, where the phenotypic effects are often large and readily discernable (*56*).

Two fairly recent developments make QTL mapping quite feasible in laboratory species—the technology to generate high-density marker maps and improved statistical methodology for data analysis. Before 1980, genetic mapping efforts relied principally on a sparse collection of morphological and histocompatibility marker loci. In the 1980s, restriction fragment length polymorphisms (RFLPs) and, more recently, simple sequence length polymorphisms (SSLPs) or microsatellites, which can be genotyped with the polymerase chain reaction (PCR), have resulted in a several-fold increase in the number of marker loci mapped in virtually all species studied (*56–58*). Because the detection and mapping of QTLs often involves sophisticated variants of linear statistical models, improved methods have aided QTL mapping primarily by increasing the power to detect and map QTLs from backcross or intercross data while taking proper account of the false positives (type I errors) expected when many markers are used to screen the genome (*57, 59*). These developments were exploited initially in plants (*56, 60*) and more recently in animals. Two or more QTLs influencing seizures (*61*) and diabetes (*62*) in mice and blood pressure in rats (*63*) were recently mapped with large segregating F_2 or backcross populations.

Recombinant Inbred Strains

Recombinant inbred (RI) strains are the fully inbred descendants of an F_2 intercross between two inbred strains (*23*). Bailey (*23*) and Taylor (*64*) realized the power of RI strains for mapping qualitative (single gene locus) effects, and early efforts identified a few such loci for drug-related traits (*12, 65*), such as the ethanol activity modifier locus (*Eam*) mapped to chromosome 4 using RI, backcross, and congenic strain data (*66*). Plomin, McClearn, and colleagues (*67*) adapted a version of the QTL mapping approach for use in existing RI strains with relatively large strain numbers. They focused principally on the BXD RI set of 26 strains derived from the C57BL/6J and DBA/2J inbred strains (*64*). By examining the pattern of strain differences in the BXD RI strains and referring the results to a database comprising the genetic map location of many marker loci (over 1200 at present), it is possible from the behavioral phenotype (the means of each strain) to infer the provisional chromosomal localization of the underlying genes without the need for new genotyping. This allows meaningful progress toward the detection and mapping of QTLs influencing many phenotypes of interest. Any RI QTL result ultimately requires subsequent confirmation testing in an F_2 intercross between C57BL/6J and DBA/2J strains, congenic strains, or other independent genetic models. Thus, the BXD RI strains can be very useful as the first of a two-step QTL mapping approach (*68, 69*).

Mapping of QTLs Affecting Drug Withdrawal Hyperexcitability

We have recently used a two-step QTL mapping approach to confirm the chromosomal location of two QTLs, provisionally termed *Aw1* and *Aw2*, that influence acute alcohol withdrawal severity in mice. By testing the BXD RI strains, we initially identified five provisional QTLs. One of these provisional QTLs (*Aw1*) in the *Pmv-7/D2Mit9* region of chromosome 2 [38 centimorgans (cM) from the centromere] accounts for about 40% of the total genetic variance in acute ethanol withdrawal and was associated with the severity of withdrawal from both ethanol and nitrous oxide (*70, 71*). Linkage between *Aw1* and the marker *D2Mit9* was subsequently shown in a population of F_2 mice derived from C57BL/6J and DBA/2J parental crosses:

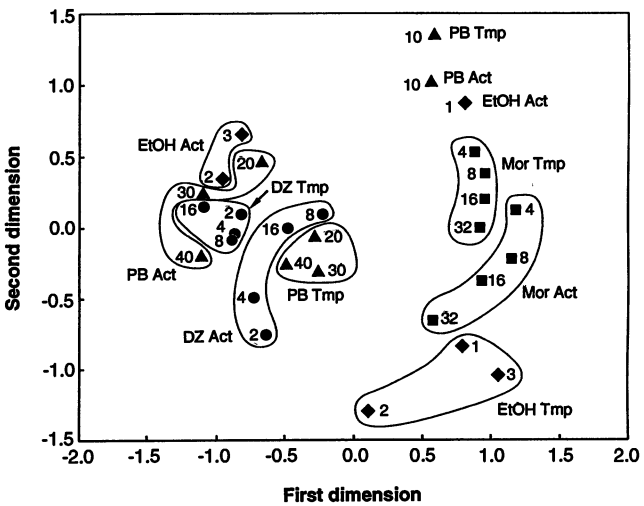


Fig. 1. Multidimensional scaling plot for genetic associations among drug-response traits. Experimentally naïve mice from 15 standard inbred strains were given one of three to four intraperitoneal doses of either ethanol (EtOH) (*90*), diazepam (DZ) (*91*), pentobarbital (PB) (*92*), morphine (Mor) (*93*), or saline, and their locomotor activity (Act) was monitored for the next 15 min. Body temperatures (Tmp) were taken 30 min after injection. Strain mean values for the saline groups were subtracted from the concurrently tested drug groups for each dose. A correlation matrix among strain means (containing 435 genetic correlations) was constructed and subjected to least squares multidimensional scaling (*94*) to reduce the original 30 variables to only two dimensions for plotting purposes. The *x*-axis shows factor loadings on the first principal component (dimension) extracted from the data, and the *y*-axis shows loadings on the second principal component. Both are linear and orthogonal combinations of the 30 variables and were chosen because they together account for the maximum proportion of the genetic variance in this data set, 85%. Linear distance represents similarity among the plotted variables in the pattern of inbred strain (genetic) differences. Points close together share common genetic influences, that is, are genetically correlated, whereas points far apart do not. The numbers near each point refer to the dose in milligrams per kilogram of body weight (or grams per kilogram body weight in the case of EtOH). Perimeters are drawn to emphasize doses from a single drug. Symbols: ◆, EtOH; ●, diazepam; ■, morphine; and ▲, pentobarbital.

that is, genotype at the marker locus *D2Mit9* significantly predicted ethanol withdrawal severity in individual mice. A second locus (*Aw2*) more distal on chromosome 2 (68 cM) confers protection against acute ethanol withdrawal; linkage of this locus to *D2Mit17* and *D2Mit58* was also confirmed in the same F_2 mice (*71*, *72*). The *Aw1* region is syntenic (homologous) with a region of human chromosome 2, which suggests that there may be a human equivalent to this QTL near 2q24–q37. Plausible candidate genes near *D2Mit9* include *Gad-1*, encoding glutamic acid decarboxylase, which catalyzes synthesis of the inhibitory neurotransmitter GABA. A cluster of nearby genes (*Scn1a*, *Scn2a*, and *Scn3a*) encode the α-subunit of brain voltage-dependent sodium channels, which are responsible for the rapid rising phase of the action potential in neurons and other excitable cells. A possible candidate gene for *Aw2* is *mEaat2*, coding for a transporter for excitatory amino acids such as glutamate (*73*).

The Cumulative Power of QTL Analyses

QTL analysis is clearly a useful hypothesis-generating approach to identify genes influencing drug sensitivity in animal models, which then may be related to homologous map sites in humans. This approach is a valuable genetic tool in cases where the relevant genes are unknown, which is typical of most drug responses. For example, QTL analysis in a set of RI strains derived from crosses between LS and SS lines has provisionally identified a region of chromosome 11 that contains markers significantly associated with ethanol sleep time (*74*). The gene coding for a PKCα maps nearby (68 cM).

In addition to providing a guide to the potential influence of individual loci on a traitwise basis, the QTL database from the BXD RI strains is cumulative by virtue of full inbreeding. Thus, the genotypes involved are stable and replicable, and data from them can be meaningfully compared from different laboratories and over time. The pattern of recombination occurring early in RI strain development has been virtually "frozen" in place by the inbreeding process. Through linkage mapping, the positions of many candidate genes, such as those encoding proteins important for neurotransmission, have also been identified. It is now becoming possible to explore the genetic landscape developed from the integration of the map locations of candidate genes with the cumulative phenotypic QTL information. Many investigators have generously provided us with preliminary data that we have used to construct an exploratory map showing provisional QTLs affecting a variety of responses to several drugs of abuse. We have included locations for candidate genes likely to be of interest in determining drug responses (*75*).

Figure 2 indicates that marker loci significantly associated ($P < 0.01$) with provisional drug-response QTLs are not randomly distributed across the mouse genome. Chromosomal regions associated with multiple phenotypes can be identified. Most of the QTL sites shown have not yet been subjected to confirmation testing and are thus provisional: our experience suggests that about 75% should be verified, but 25% are likely to be the result of type I error. The purpose of plotting them is simply to explore the possibilities for synthesis of information that should be attainable in the near future as a result of the cumulative nature of RI strain data.

Two experiments in different laboratories have analyzed voluntary ethanol preference drinking in the BXD RI mice. Of the five chromosomal regions identified for preference in one laboratory (red 7, chromosome:cM locations 2:49, 7:13, 7:51, 4:59, and 9:28), the first three were also identified in the other laboratory (red 3) (Fig. 2). This apparent convergent validation of the method suggests that the findings are substantially replicable in different laboratories.

Other chromosomal regions of clear interest ("hotspots") can also be discerned. Several responses to alcohol (sensitivity to ataxia, tolerance to hypothermic and ataxic effects, preference drinking, and conditioned place preference) indicate the effects of QTLs influencing all these phenotypes on the middle portion of chromosome 9 (red 7, 11, 12, 14, and 15 at 9:27–31 cM). Four of the five traits showed their highest association with the same marker, *Cyp1a1*, at 9:31. This strongly suggests, but does not prove, that a single locus accounts for all these associations (*76*). Consumption of methamphetamine (in saccharin), methamphetamine-stimulated activity, and haloperidol-induced catalepsy also map to this region, and morphine-induced Straub tail maps nearby (9:20). This region is of interest because of the presence of a logical candidate gene, namely, the gene coding for the dopamine D2 receptor (*Drd2*). Dopamine is an important neurotransmitter in the reward pathways of the brain, as well as in those pathways serving forward locomotion (*77*). The ethanol preference (*78*) and haloperidol catalepsy (*79*) associations with markers near *Drd2* have been verified in F_2 mice with PCR genotyping. A significant genetic correlation between dopamine D2 receptor density and catalepsy in standard inbred mouse strains further suggests control of haloperidol catalepsy by dopamine D2 receptors (*79*).

Another region of interest spans 32 to 45 cM on chromosome 6, where QTLs were identified for both chronic alcohol and nitrous oxide withdrawal. Three other alcohol responses, two responses to methamphetamine, saccharine preference drinking, and haloperidol catalepsy, were also associated with this region. However, no apparent candidate gene or genes are currently mapped to this region. The acute ethanol withdrawal protective locus located near 68 cM on chromosome 2 discussed above also was associated with ethanol hypothermic sensitivity and ethanol-conditioned taste aversion. Other apparent hotspots were revealed on chromosome 1 (43–53 cM and 83–102 cM), chromosome 2 (80–86 cM), chromosome 4 (21–27 cM), and chromosome 8 (15–25 cM).

A final example of the potential interest of this synthetic approach is a cluster of four ethanol, one morphine, and one cocaine responses mapping to chromosome 9 (42–48 cM). The gene coding for the 5HT1B serotonin receptor maps to 9:40 cM. It would be interesting to test the recently developed transgenic mice lacking 5HT1B receptors (see below) for the drug response traits mapped to this region.

In recent years, there has been considerable progress in mapping genes that control various inherited human disorders, and in several instances this has led to successful positional cloning of the responsible gene (*80*). However, these successes have largely been restricted to single-gene mutations, which are relatively rare in the absence of chemical mutagenesis (*8*), whereas the genetic control of susceptibility to many common disorders, such as substance abuse, is multigenic and hence more difficult to analyze (*81*). One way to approach this problem is to map the genes relevant for the studied trait first in the mouse and subsequently use the information about homology of chromosomal regions between mouse and human to study the relevant region in humans (*82*).

Human association studies are focused on the identification of risk markers. The relative rarity of individuals with disease or drug abuse traits makes it very difficult to identify specific markers whose linked genes might provide protection, for such markers could be present in the vast majority of individuals. An advantage of QTL mapping in animal models is the ability to locate protective as well as risk markers. The more distal acute ethanol withdrawal locus on mouse chromosome 2 appears to represent such a protective marker (*71*, *73*); syntenic correspondence to human gene regions suggests a homolog might be found on human chromosome 11p or 15q.

From Marker to Gene

QTL analysis promises to identify the chromosomal position of many genes influencing quantitative traits such as those reviewed

above. The recent development of dense genetic maps allows definition of the location of the gene or genes of interest within a few million base pairs, and it represents the first step in characterizing and cloning the underlying genes. Positional cloning of human disease genes has demonstrated that even when the position of a gene has been defined within one or two million base pairs and all the DNA sequences within that region have been isolated, identification of the relevant gene can still be a formidable task. The recent success of transgenic technologies employing yeast artificial chromosomes (YAC transgenics), and more traditional approaches employing congenic strains, promise to bridge the gap between cloning and behavior (*8*).

Transgenic Animal Models

Transgenic technology creates a very effective tool for analyzing the physiological roles of specific genes (*8*). Low and colleagues have recently employed a "knockout" strategy, using homologous recombination to disrupt the pro-opiomelanocortin gene and produce transgenic mice incapable of synthesizing β-endorphin, a neuropeptide that may influence drug sensitivity and other behaviors (*83*). Similarly, Hen and colleagues have produced a null mutant mouse totally devoid of serotonin 5HT1B receptors (*84*). Although the 5HT1B knockout animals have normal appearance, normal locomotor activity under many conditions, and respond normally in one test of anxiety, they are markedly more aggressive than their nontransgenic littermates. Thus, the role of 5HT1B receptors in the drug responses suggested to be associated in Fig. 2 (for example, cocaine activation and ethanol-conditioned taste aversion) can be tested directly. In addition to facilitating the study of known candidate genes, molecular complementation (transfer of specific genes) of selected phenotypes is a potentially important tool for gene identification. In particular, YAC transgenic technology holds great promise for studying QTLs that influence a developmentally restricted phenotype, which requires the transfer of both the locus and the long-range regulatory element or elements responsible for normal temporal or regional expression of the gene.

Congenic Strains and Representational Difference Analysis

Classical transmission genetics can also be used to transfer a gene of interest from a donor strain or mutant onto the genetic background of an inbred strain. Through successive generations of backcrossing and selection, it is possible to produce congenic strains that are genetically identical except in a small region surrounding the gene of

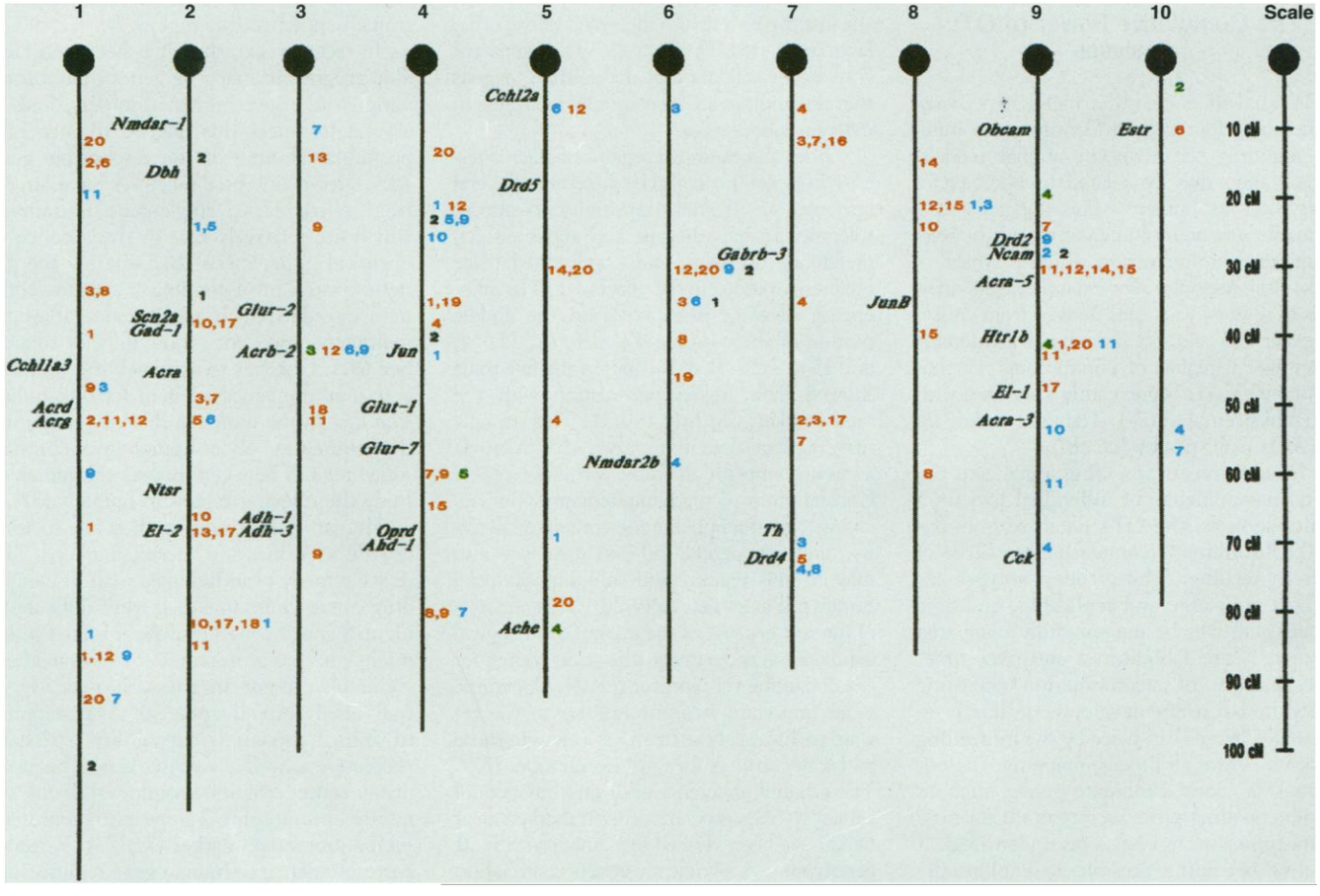


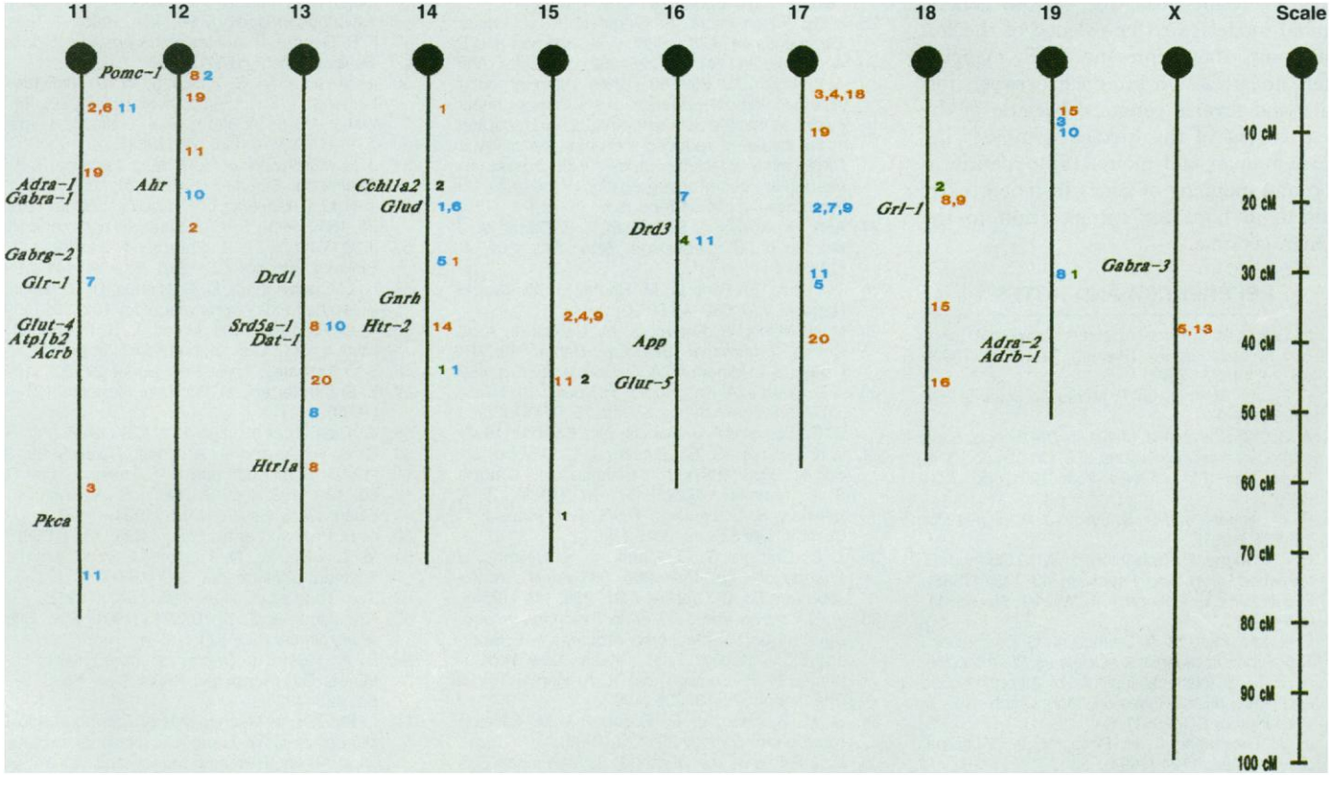
Fig. 2. Schematic representation of genomic locations of provisional quantitative trait loci (QTLs) affecting drug responses. The 19 mouse chromosomes and the X chromosome are represented proportionately: scale gives centimorgans (cM) from the centromere (represented as large dots at apex). Each centimorgan of distance between two markers represents a 1% recombination probability. Numbers represent drug-response traits. Number color represents drug [red = ethanol, blue = methamphetamine (cocaine), green = morphine, black = other]. Number position indicates the location of a provisional QTL detected for that trait. QTLs are typically detected as clusters of significant linked markers: position of the most highly correlated mapped marker is indicated, using centimorgan distances from (*95*), except for chromosome 7, where distances are derived from L. J. Maltais, D. P. Doolittle, T. H. Roderick, A. L. Hillyard, and M. T. Davisson [*Mouse Genome* **92**, 62 (1994)]. All provi-

interest (*23*). By selection of individuals with highly responsive phenotypes for backcrosses, this procedure has been used to transfer genetic regions leading to high sensitivity to ethanol-induced activity from genetically segregating mice onto the low-activation C57BL/6 genotypic background (*85*). However, most applications of congenics will be based on genotypic rather than phenotypic markers.

The differential region of congenic strains can be small enough to permit chromosome walking to the target gene but large enough to contain RFLPs. Genetically directed representational difference analysis (GDRDA) is a new technique designed to identify previously uncharacterized differences between two DNA samples that are genetically identical except in the region of interest (*86*). This subtractive technique is unique in that it targets and reveals polymorphic markers linked to a particular trait without prior knowledge of their biochemical function. It has been used to locate several genetic markers within a few centimorgans of the mouse tottering (*tg*) and pudgy (*pu*) loci using congenic strains. GDRDA may also be applied to crosses where sequential subtractions between recombinant F_2 progeny, identified with flanking markers, target successively smaller intervals (*86*). This approach has located several genetic markers that map within 0.2 cM of the mouse nude (*nu*) locus, that is, a region representing about 200 kb or about 0.05% of the mouse genome. The ultimate resolution of this approach should be limited only by the actual density of polymorphisms detectable by GDRDA, estimated to be 1 to 2 per megabase. We have recently begun to develop congenic strains that should allow positional cloning of QTLs influencing several traits, including the severity of withdrawal from ethanol and other depressants, ethanol preference, and ethanol-conditioned place preference. Mice possessing the desired alleles at SSLP markers flanking the QTL of interest are chosen for mating. Using this approach, we will be able to transfer regions containing risk or protective QTLs, or multiple QTLs, onto appropriate background strains.

Antisense Oligodeoxynucleotide Strategies

Antisense strategies provide a simple method for down-regulating specific mRNAs and their proteins at any stage of development with a high degree of selectivity. The careful design of antisense oligonucleotides that interact with specific RNA species (for example, those encoding a candidate protein) can be used to decrease translation of the protein. They have been effectively used to demonstrate the importance of the γ_2 subunit in mediating sensitivity of the $GABA_A$ receptor to diazepam and ethanol in vitro (*18*). In vivo studies with intracerebroventricular infusion or repeated injection of antisense oligonucleotides have proven successful in reducing brain neuropeptide Y1, dopamine D2, and NMDAR1 glutamate receptor levels and for examining their role in behavior. Importantly, this approach was successfully used with NMDAR1, although a "knockout" of this gene by homologous recombination was lethal (*87*). This demonstrates the complementary nature of antisense and transgenic approaches. In recent studies, intrathecal administration of δ-opioid antisense oligonucleotides to mice produced a selective and reversible loss of δ-opioid analgesia and receptor binding, whereas microinjection of μ-opioid antisense into



sional QTL associations shown were significant at $P < 0.01$. Candidate gene names are given in italic black to the left of each chromosome (for example, *Acrg* represents the mapping of the gene coding for the acetylcholine receptor γ chain to 52 cM on chromosome 1), using standard nomenclature given in (*95*). For a complete list of candidate gene names and a list of drug-response traits, with reference indicating source of data, see (*96*). Approximately 40% more provisional QTLs were indicated when a criterion value of $P < 0.05$ was used (*97*). All provisional QTLs represented are the results of initial screening of BXD RI strains. They should be considered provisional until they can be verified by additional testing of genetically segregating populations (for example, F_2 or backcross populations). On the basis of our confirmation-testing experience, it is predicted that 75% of these provisional QTLs would be verified by such additional testing.

the periaquaductal gray completely blocked the analgesic actions of locally administered morphine (*88*). Antisense injections into parenchyma have also been effective in other brain regions, although site injections may be more likely to cause oligodeoxynucleotide toxicity and tissue damage (*89*). These studies clearly illustrate the utility of antisense strategies for elucidating the molecular mechanisms of drug sensitivity and other behaviors.

Conclusions

Selective breeding studies have made significant progress in identifying neurobiological mechanisms underlying certain drug responses, and their use will likely continue to be important in this area of research. The systematic analysis of inbred strain databases is beginning to reveal features of the genetic landscape describing several abused drugs and their effects. Past work has documented the influence of anonymous genes on drug responsiveness. The future will increasingly move toward identification, mapping, cloning, and characterization of particular genes. Behavioral and molecular genetics are being combined to integrate results from the various genetic animal models with knowledge of the human traits they represent. QTL mapping offers an attractive interface between forward and reverse genetics: because of the great extent of the linkage homology between human and mouse (*82*), identification and mapping of genes in mouse offers immediate hope for extrapolation to the human genome.

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Candidate gene names are given in bold italics to the left of each chromosome. *Ache*, acetylcholinesterase; *Acra*, *a-3*, *a-5*, *b*, *b-2*, *d*, and g, acetylcholine nicotinic receptor, α, α-3, α-5, β, β-2, δ, and γ subunits; *Adh-1* and *-3*, *Ahd-1*, alcohol dehydrogenases; *Adra-1*, *a-2*, *and b-1*, adrenergic α-1, α-2, and β-1 receptors; *Ahr*, aryl hydrocarbon hydroxlase inducibility; *App*, Amyloid β plaque protein; *Atp1b2*, Na^+,K^+-ATPase, β-2 subunit; *Cchl1a2*, *1a3*, *and 2a*, dihydropyridine-sensitive (L-type) calcium channel subunits 1A2, 1A3, and 2A; *Cck*, cholecystokinin; *Dat-1*, dopamine transporter; *Dbh*, dopamine beta hydroxylase; *Drd1 to Drd5*, dopamine D1 to D5 receptors; *El-1* and *-2*, mouse epilepsy loci 1 and 2, see (*61*); *Estr*, estrogen receptor; *Gabra-1*, *a-3*, *b-3*, *and g-2*, gamma aminobutyric acid receptor, α1, α3, β3, and γ2 subunits; *Gad-1*, glutamic acid decarboxylase; *Glud*, Glutamate dehydrogenase; *Glr-1*, *Glur-2*, *-5*, *and -7*, glutamate receptors 1, 2, 5, 7; *Glut-1 and -4*, glucose transporters; *Gnaz*, guanine nucleotide binding protein α z-subunit; *Gnrh*, gonadotropin-releasing hormone; *Grl-1*, glucocorticoid receptor; *Htr1a and 1b*, *Htr2*, serotonin 5-HT1A, 5HT1B, and 5HT2 receptors; *Jun*, *JunB*, oncogenes; *Ncam*, neural cell adhesion molecule; *Nmdar-1 and -2b*, *N*-methyl-D-aspartate 1 and 2B receptors; *Ntsr*, neurotensin receptor; *Obcam*, opioid binding protein; *Oprd*, delta opioid receptor; *Pkca*, protein kinase C α; *Pomc-1*, proopiomelanocortin; *Scn2a*, Na^+ channel, type II, α-subunit; *Srd5a-1*, steroid 5-α reductase; *Th*, tyrosine hydroxylase.
97. J. C. Crabbe, J. K. Belknap, K. J. Buck, data not shown.

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