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Modeling the Diagnostic Criteria for Alcohol Dependence with Genetic Animal Models

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Abstract

A diagnosis of alcohol dependence (AD) using the DSM-IV-R is categorical, based on an individual's manifestation of three or more symptoms from a list of seven. AD risk can be traced to both genetic and environmental sources. Most genetic studies of AD risk implicitly assume that an AD diagnosis represents a single underlying genetic factor. We recently found that the criteria for an AD diagnosis represent three somewhat distinct genetic paths to individual risk. Specifically, heavy use and tolerance versus withdrawal and continued use despite problems reflected separate genetic factors. However, some data suggest that genetic risk for AD is adequately described with a single underlying genetic risk factor. Rodent animal models for alcohol-related phenotypes typically target discrete aspects of the complex human AD diagnosis. Here, we review the literature derived from genetic animal models in an attempt to determine whether they support a single-factor or multiple-factor genetic structure. We conclude that there is modest support in the animal literature that alcohol tolerance and withdrawal reflect distinct genetic risk factors, in agreement with our human data. We suggest areas where more research could clarify this attempt to align the rodent and human data.

Keywords

Withdrawal; Tolerance; Genetic correlations; Gene expression

1 Introduction

In the US, alcohol dependence (AD) is diagnosed using criteria set out in the Diagnostic and Statistical Manual-IV-R (DSM-IV-R) of the American Psychiatric Association. Seven criteria are evaluated, and if an individual displays any three or more of them during the same 12-month period, he or she meets the diagnostic criteria. The seven criteria are:

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tolerance (more must be drunk to achieve the desired effect, or drinking the same amount produces a much diminished effect), withdrawal (symptoms appear when drinking is discontinued), loss of control (drinking cannot be modulated once started), desire to quit (drinking is initiated despite desire not to drink), preoccupation (excessive thoughts and activities pertaining to gaining access to or consuming alcohol), activities curtailed (other activities are reduced as time is dominated by drinking and recovering), and persistence (drinking continues despite medical and/or social consequences). The other diagnostic scheme in wide use, the International Classification of Diseases (ICD)-10, is similar in its categorical approach to diagnosis and congruence between diagnoses using the two systems is high.

There is a substantial genetic contribution to risk for AD. Twin, adoption and other studies suggest that 50–60% of individual differences in risk are heritable (Enoch and Goldman 2001; Goldman and Ducci 2007). However, there is no consensus on whether AD diagnosis represents a single versus a multifactorial genetic phenotype. Some studies suggest that there are different subtypes of alcoholism with distinct genetic architectures, with many possible subtyping schemes proposed [e.g. (Cloninger 1987)]. Recently, the contributions of the individual DSM-IV-R criteria have been suggested to represent a single continuum of underlying risk (Grant 2000), perhaps primarily reflecting heavy consumption (Grant et al. 2009). Most gene finding studies have used the categorical diagnosis of AD, assuming that the linkage or association of variation in candidate genes or genetic markers is generally informative for diagnostic risk [e.g. (Edenberg and Foroud 2006; Prescott et al. 2006; Treutlein and Rietschel 2011)]. Many individuals have positive scores on more than one of the seven diagnostic criteria for AD. In one epidemiological study, one-fourth of AD individuals had positive scores on 5, 6, or all 7 criteria (Grant 2000).

Genetic contributions to individual differences in the degree of responses related to alcohol have been studied in animal models for many years. It was established in the 1940s that genes led some rats to prefer to drink a 10% alcohol solution more than others (Mardones and Segovia-Riquelme 1983) and in 1959 that different inbred strains of mice had genetic proclivities ranging from strong preference (such as the C57BL/6J strain) to near abstinence (such as the DBA/2J strain) (McClearn and Rodgers 1959). Genetic animal models have been a major influence on alcoholism research since then, and their contributions have been reviewed elsewhere (Crabbe 2008; Edenberg and Foroud 2006). Some genotypes of rats and mice have been developed through selective breeding for high two-bottle ethanol preference drinking. Preferring (P), Alko Alcohol (AA) and several other rat lines (and High Alcohol Preferring—HAP—mouse lines) drink a substantial amount of alcohol during each 24 h day, especially as compared with NP (ANA, LAP and others) selected for low drinking (Crabbe et al. 2010b). Some argue that the genetic high preferrers represent an animal model of alcoholism (Bell et al. 2006; Sommer et al. 2006). However, they do not usually drink enough to become intoxicated at any point during the day. Nor do they show pronounced tolerance or withdrawal after voluntary drinking. Thus, we have argued that these selected lines of animals do not constitute a model of the complex AD diagnosis, but rather capture genetic contributions to select aspects of alcohol drinking (Crabbe 2008; Crabbe et al. 2009). If genetic animal models cannot capture the entire range of such a complex genetic trait as AD, a more reasonable goal when designing such a model is to target specific alcohol-related responses (McClearn 1979); indeed, this has been the practical goal followed during the development of each such model. Rodent lines have also been selected for high versus low genetic susceptibility to alcohol intoxication/sedation, locomotor stimulation, tolerance, and withdrawal severity (Browman et al. 2000; Crabbe 2008).

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2 Goal of the Review and Method of Analysis

The goal of this paper is to review how data from rodent models inform the debate regarding whether AD represents one or multiple underlying genetic factors. Specifically, can the various rodent genotypes that have been used to study alcohol's effects provide evidence for or against the single-factor AD risk hypothesis? In a recent analysis of twin data (see Sect. 3), we found that the diagnostic criteria for DSM-IV-R AD diagnosis represent three somewhat distinct genetic paths to individual risk (Kendler et al. in review). If an animal known to be genetically susceptible to one effect of alcohol also proves to be genetically susceptible to another, this suggests that the two traits may share common genetic determinants—i.e. are genetically correlated. Thus, in genetic terms, we will review the animal evidence for genetic correlation, where genes exert pleiotropic effects on multiple phenotypes. Specifically, if a rodent that is genetically high-scoring on one criterion of an AD diagnosis also scores high on many or most others, we would take this as evidence favoring a single genetic factor model for the construct. An informative rodent study would need to be able to discriminate a genetic from an environmental source for that correlation. Alternatively, if the pattern of genetic influences across criteria in rodents paralleled the human data, we would take this as supporting the multiple factor model. In the twin paper, we suggested that the animal data provided partial support for the human genetic architecture (Kendler et al. in review). Here, we discuss the animal data in more depth.

In this review, we first discuss the behaviors themselves and the similarities and differences between the human criteria and their counterpart animal assays. The degree to which rodent behaviors are consilient with the human symptoms they attempt to model presents a difficult problem (Cicero et al. 1979; Crabbe 2010). We then describe the most powerful animal genetic methods available for comparing the animal and human genetic data. We next explore the genetically informative animal data in more detail. Finally, we review the most relevant genetic data from animal models based not on the allelic differences among individuals, but on the differential expression of genes. We conclude that the three-factor model for the human data is broadly consistent with the majority of the animal data. We also identify areas where there could be substantial improvements made in providing discrete models for some of the human diagnostic criteria, and give examples of how those new models could be used to test the hypothesis further.

3 Three Distinct Clusters of Genetic Risk Influence Alcohol Dependence Diagnosis on DSM-IV-R

In a recent multivariate twin analysis of interview data from 7,548 adult twins from the Virginia adult twin study of psychiatric and substance use disorders, we used structural equation modeling to clarify the structure of the genetic and environmental risk factors for each of the seven individual criteria for AD diagnosis. Also included in the model were two screening items, positive response to which was necessary for entry into the alcohol section of the interview. The best fit model included three genetic common factors, two unique environmental common factors and environmental factors unique to each criterion (Kendler et al. in review). We termed the genetic risk factors: (a) heavy use and tolerance (loading on the first screening item reflecting excess quantity or frequency of alcohol consumption and the tolerance criterion); (b) loss of control with alcohol associated social dysfunction (which loaded heavily on loss of control, desire to quit, preoccupation, and activities given up); and (c) withdrawal and continued use despite problems. We do not consider the environmental risk factors here.

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4 Consilience of Animal Phenotypes and Human Diagnostic Criteria

Of the seven criteria for an AD diagnosis in DSM-IV-R, not all can reasonably be modeled in rats or mice. It has been realized for many years that the principal strength of rodent genetic animal models is to produce partialmodels for complex human traits (McClearn 1979). Research into the genetics of alcoholism is rich in both human and rodent data, but most researchers work with humans only, or with one or the other rodent species. Perhaps as a consequence, the behaviors studied in rodent laboratories often do not completely resemble their human counterparts. A recent effort to address the problems of better consilience between human behaviors and laboratory rodent behavioral targets focused on alcohol-related traits (Crabbe 2010) and considered several features of human alcoholism in detail, comparing several aspects of risk and comorbidity. The reviews resulting from this effort also focused on the genetic correlations among different traits (Ehlers et al. 2010; Stephens et al. 2010; Sher et al. 2010; Crabbe et al. 2010a; Heilig et al. 2010; Dick et al. 2010; Leeman et al. 2010).

We consider first each of the seven criteria and discuss whether the rodent behavioral assays plausibly parallel the human diagnostic criterion. We limit the animal discussion to the data from rodents, as they comprise the majority of the genetic animal model work. Desire to quit, preoccupation, and loss of control have not been modeled in rodents, and we believe it unlikely that a rodent parallel for these self-report measures exists. Similarly, “activities given up” has not been modeled directly. Continued use despite problems could perhaps be approached through certain animal behavioral assays, but no relevant genetic data currently exist to our knowledge. Thus, we set aside these five of the seven criteria. Withdrawal and tolerance have clear laboratory animal parallels. Given the clear distinction from the human data between withdrawal and tolerance, the preponderance of relevant data from animals is adduced to trying to ascertain whether the animal data support commonality of genetic influence or lack thereof on tolerance and withdrawal.

4.1 Tolerance

Drug tolerance is defined as the reduction of response intensity or duration after chronic administration; alternatively, it is defined as the requirement to raise the dose of a drug in order to maintain an initial level of response (Kalant et al. 1971). There are two mechanistically distinguishable types of tolerance, pharmacodynamic (functional) and pharmacokinetic (metabolic). In functional tolerance, the amount of drug and/or active metabolite that remains in contact with the effector tissue has not changed, but the target tissue no longer responds in the same way. For example, we assume that many of alcohol’s behavioral effects are due to interactions with brain, and in a tolerant individual, certain receptors may no longer signal their intracellular partners as effectively. Metabolic tolerance occurs when the processes of drug absorption, distribution among body compartments (e.g., brain, blood, soft tissues), metabolism to other chemicals, and/or excretion lead to a significant reduction in the amount of alcohol in the body—specifically, at the effector tissue. Given alcohol’s pharmacokinetics, this can occur if alcohol’s metabolic enzymes, primarily alcohol dehydrogenases (ADH) and aldehyde dehydrogenases (ALDH), have been induced to work more actively.

Both types of tolerance occur with alcohol. For humans, alcohol metabolism is involved with risk of AD. Polymorphisms in ADH and ALDH enzymes have provided the clearest evidence of an individual gene’s important role in risk for an AD diagnosis. Many individuals of East Asian descent possess polymorphisms in ALDH that lead to slow elimination of acetaldehyde, a toxic metabolite. The circulating acetaldehyde in turn causes symptoms including facial flushing, nausea, dizziness, and headache, and these individuals have a clear lowered risk of developing AD (Chen et al. 1999; Enoch and Goldman 2001).

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However, the development of metabolic tolerance (i.e., more rapid elimination of alcohol with chronic use) is not thought to be an important factor in progression to an AD diagnosis.

Functional tolerance, on the other hand, plays a role in humans and is one of the diagnostic criteria for AD. The DSM-IV-R criterion for tolerance is typically assessed with two questions similar to the following: (1) Did you ever find that you needed to drink a lot more in order to get the same effect as you did when you first started drinking? And (2) Did you ever find that when you drank the same amount it had much less effect than before? Follow-up questions would then assess the actual amount of increased alcohol required to get the "same effect."

One way that tolerance has featured in analyses of genetic risk factors derives from the findings of Marc Schuckit's group and others beginning in the early 1980s. Family history positive (FHP) individuals were known to be at greater risk for AD than Family history negative (FHN) subjects. Schuckit's group brought young FHP and FHN men into the laboratory and gave them an alcohol challenge. He found that FHP subjects reported less sedation, body sway and felt less "high" than FHN subjects. They also showed blunted hormone responses. Following these subjects over the years revealed that so-called "low level of response" to alcohol predicted eventual AD diagnosis even better than FHP versus FHN status (Schuckit and Smith 1996; Schuckit 2000). However, the pattern of lower level of response in FHN subjects was not always seen by other investigators. A review of the literature suggested that so-called "low level of response" probably depends upon when the measurement is taken. If taken early after alcohol administration, FHP subjects actually show *enhanced* responses to alcohol relative to FHN for some measures, but if assessed an hour or more after ingestion, FHP responses tend to be lesser than FHN (Newlin and Thomson 1990). This interpretation suggests that low level of response actually represents the more rapid development of acute functional tolerance (AFT) during the test session by FHN subjects.

In rats and mice, substantial metabolic tolerance does not normally occur if animals are drinking alcohol chronically unless there is no water available. Several procedural manipulations can be performed that increase rodents' oral intake of ethanol solutions, but these are typically labor-intensive and many require weeks if not months of access before animals will drink enough alcohol that they will display metabolic tolerance. Thus, differences in metabolic tolerance to ethanol are not normally a consideration for interpreting most animal studies.

To produce functional tolerance, rodents need to be given repeated injections or gastric intubations of alcohol. To produce greater levels of functional tolerance, animals may be fed a liquid diet, where alcohol solutions with added vitamins and minerals are substituted for food and water. Alternatively, they may be exposed to alcohol chronically by being placed in a chamber where alcohol vapor is provided and thus be chronically dosed by inhalation.

To measure functional tolerance in rodents, the usual practice is to study a sedative or intoxicating response. Although it is possible to measure tolerance as the increase in dose required to maintain a given level of intoxication, this has rarely been done for sedating drugs [but see (Okamoto et al. 1978)] and usually tolerance is indexed as the attenuation of the initial response (e.g., motor impairment, hypothermia, depression of rate of operant responding).

Functional tolerance can be further subdivided into three types based on the duration and/or frequency of alcohol exposure. Chronic tolerance is seen with multiple injections or other exposure regimens. It used to be thought that functional tolerance to alcohol took days or

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weeks of repeated or continuous exposure to develop (Kalant et al. 1971), but we now know that it can develop more quickly.

At the other extreme, acute functional tolerance was first reported by Mellanby (1919) who studied dogs walking on a treadmill while implanted with a jugular catheter. He infused alcohol and recorded the blood alcohol level at which the animals first began to stumble and drag their feet. After a period, he discontinued the infusion, and recorded another blood alcohol level when the animals first regained the ability to walk without stumbling. The recovery alcohol level was higher than the initial value, indicating that a higher dose was necessary to produce intoxication at the later time point, which suggests the existence of AFT (as no measures were taken of brain alcohol levels, metabolic tolerance could not be ruled out). AFT has since been demonstrated in mice by comparing blood alcohol levels at recovery and loss of function (ability to remain balanced) on a rod (Gehle and Erwin 2000). AFT is the type of tolerance apparently shown by Schuckit's FHP subjects, as it apparently occurs within a single alcohol dosing session.

Bridging the gap between AFT and chronic tolerance, mice (Crabbe et al. 1979) and rats (Khanna et al. 1991) have shown a third type of tolerance, rapid tolerance, where response to a second injection of alcohol is reduced from the initial response. While chronic and rapid tolerance appear to be similar mechanistically, this is less certain for AFT, which may represent a unique adaptation (Kalant 1998).

4.2 Withdrawal Severity

The occurrence of withdrawal symptoms when a drug is discontinued is interpreted to mean that a state of dependence on the drug was present (Kalant et al. 1971). While some suggest that physical and psychological dependence are distinguishable entities, we do not see how this distinction can easily be made. For alcohol dependence, it has long been known that a range of withdrawal symptoms appear with characteristic temporal waxing and waning severity (Victor and Adams 1953; Isbell et al. 1955). Alcohol withdrawal symptoms include irritability, nausea, vomiting, tremor, anxiety, insomnia, hyperthermia, hyperventilation, tachycardia, and central nervous system hyperexcitability manifested as convulsions, seizures, hallucinations, and delusions (Metten and Crabbe 1996). The core symptoms are remarkably conserved across species that have been studied with certain species-specific exceptions [e.g., rodents cannot vomit; hallucinations and delusions would be difficult to document in rodents; rodents show numerous behavioral symptoms that are not extensively documented in other species (Friedman 1980)]. As with tolerance, withdrawal can be acute or chronic. For humans, acute withdrawal usually refers to symptoms that occur early after drinking ceases, and later symptoms may be described as protracted withdrawal or abstinence (Heilig et al. 2010).

Assessing the DSM-IV-R criterion of withdrawal is typically done by asking a question such as the following after showing the respondent a page full of symptoms including "the shakes," "trouble sleeping," "feeling anxious," "heart beating fast" etc.: After cutting down or stopping drinking did you ever experience any of these problems? Follow-up questions would then assess the number and duration of the withdrawal symptoms.

Alcohol dependence is typically induced in rodents using the liquid diet or vapor inhalation procedures described in the previous section. Occasionally multiple injections or intubations of the drug are given. For mice, the most frequently studied behavioral index of withdrawal severity is the handling-induced convulsion, or HIC (Goldstein and Pal 1971). This sign ranges from a mild myoclonus through clonic convulsions and if an animal is severely dependent, it may show lethal tonic hindlimb extensor seizures. Severity of withdrawal is a joint function of alcohol dose and duration of exposure (Goldstein 1972) and the symptoms

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normalize after a few days. This behavioral sign is very sensitive, and has allowed investigators to document an acute withdrawal reaction (increased convulsions) a few hours following a single high dose of ethanol (Crabbe et al. 1991). Rats do not exhibit handling induced convulsions (Heilig et al. 2010) and withdrawal severity is generally indexed by a collection of somatomotor and other behavioral and physiological disturbances (Majchrowicz 1975).

Recently, there has been a great deal of interest in the possibility that even weeks after alcohol withdrawal is initiated, behavioral signs of anxiety-like behavior may be detectable in rats (Valdez et al. 2002; Heilig et al. 2010; Pandey et al. 1999; Wills et al. 2009). While anxiety-like behavior has been reported in mice early during ethanol withdrawal, it is more difficult to document unequivocally in mice and has rarely been studied weeks after withdrawal has been initiated (for review, see Kliethermes 2005).

5 Rodent Methods for Assessing Genetic Correlation

Most behavioral traits are influenced by many genes, and usually any single gene exerts a relatively small effect on the trait. This broad genetic influence reflects the underlying biology. For example, “alcohol tolerance” is not mediated by a single neurotransmitter system and does not result from changes in a single brain area or circuit. Thus, the extremely powerful tools for manipulating single genes, including production of null mutants, gene knockdowns, viral mediated gene transfer, and transgenic over expression of a gene are unlikely to help us understand whether the total collection of genes influencing two traits are highly correlated or mostly distinct. The fact that one gene affects two traits is insufficient evidence for overall shared genetic risk. For example, nearly 100 genes have been targeted to produce null mutants or over expression transgenics, and many of these mice have been tested for ethanol preference drinking. When these studies were reviewed, the results showed that 1/3 of the genes appeared to produce a modest increase in preference drinking, 1/3 a modest decrease, and 1/3 were without effect (Crabbe et al. 2006). Thus, we do not consider the studies involving targeted genes for our assessment of genetic correlation.

Two genetic methods allow a relatively powerful assessment of genetic correlation. The first is to selectively breed lines of rats or mice for one target trait. In this laboratory analog of natural selection, breeders are chosen from the extreme responders in the population, and over generations, the selected line develops an extreme response. Usually, a parallel line is selected for low response. The genetic mechanism at work in a successful selective breeding project is that the frequencies of alleles at genes that influence the trait under selection are increased until all animals have two copies of the same allele for each relevant gene. If the selected lines are now compared for the trait postulated to be genetically correlated, and are found to differ, the most likely explanation is that the second trait is a genetically correlated response to selection. The principal limitation of this approach is that only those traits that have been selected for can be assessed, but its strength is that the potential correlated responses that can be tested are unlimited. The many methodological intricacies and caveats surrounding this approach have been discussed elsewhere (Crabbe et al. 1990).

The other relatively powerful method is to use inbred strains. Within an inbred strain of mice or rats, close relatives have been mated for more than 20 generations. The result of this inbreeding resembles that of selective breeding—gene frequencies increase and eventually become “fixed” and all animals possess two identical alleles (i.e., are obligate homozygotes) at each gene. There are however two major differences. The specific allele at each gene that is fixed in an inbred strain has no necessary relationship to any phenotype—it has been captured by chance. Second, unlike selected lines, inbred strains are homozygous for all genes (in selected lines, multiple alleles continue to segregate at all genes unrelated to the

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trait under selection). If inbred animals from a substantial number of strains are tested for two traits, their mean responses can be correlated to assess genetic correlation rather directly. The more strains that can be tested, the more powerful is the test of genetic correlation. Many studies have been performed with one specialized set of inbred strains called BXD recombinant inbred (BXD RI) strains. These resemble standard inbred strains except that they are originally derived from the intercross of C57BL/6J And DBA/2J inbred strains and therefore have a much simpler genetic structure. Only two alleles are possible at any gene, one derived from each progenitor inbred strain. Because these two progenitor inbred strains differ markedly in response to nearly all drugs of abuse, including alcohol, the BXD RI strains display a wide spectrum of responses to alcohol for nearly all traits. Their use for gene mapping has been described elsewhere (Palmer and Phillips 2002), but for the present discussion, they are a very similar tool for assessing genetic correlation of two traits. Technical details surrounding the inbred strain panel approach have been discussed elsewhere (Crabbe et al. 1990).

A final group of issues surrounds the nature of the human experiment. The human data discussed here were derived from monozygotic (identical) and dizygotic (fraternal) twins. Two individuals from an inbred mouse or rat strain are a plausible surrogate for one monozygotic twin pair (but not a perfect one—there is no heterozygosity within an inbred strain). But no dizygotic twin pair can be produced that shares the parental genetic background with an inbred strain. And although there are more than 100 available mouse standard inbred strains, and several sets of multiple RI strains, studying enough inbred strains to perform a path analysis like that presented in this article would present a host of logistical and financial challenges.

6 Evidence for Genetic Correlation Across AD Criteria in the Rodent Literature

We consider here in turn the evidence from selectively bred animal lines; from correlations among strain means for standard inbred mouse strains; and strain mean correlations from the BXD recombinant inbred strain panel. The traits studied are summarized in the *Sidebar*, and the correlations across strain means are given in Tables 1 and 2.

6.1 Selected Lines

Mouse lines have been selected for the severity of withdrawal HICs (Crabbe et al. 1985). Starting with a genetically heterogeneous stock of mice where as many as eight alleles were segregating for any locus, a large population of animals was exposed to ethanol vapor for 72 h to induce a state of physical dependence. After removal from the inhalation chambers, mice showed waxing and then waning HIC severity for up to 24 h, with peak HICs seen at about 7–10 h into withdrawal. Mice with the most severe withdrawal HICs were mated to produce the ensuing generation of Withdrawal Seizure-Prone (WSP) mice, and those with the least severe HICs were mated to initiate the Withdrawal Seizure-Resistant (WSR) line. The experiment is replicated, so there were two, genetically independent WSP (WSP-1 and -2) and WSR (WSR-1 and -2) pairs of lines generated. Each generation thereafter, each line was reproductively isolated, and the most (or least) severe-scoring mice were used as mating pairs.

By the 11th selected generation, both WSP lines had at least tenfold more severe withdrawal HICs than their respective WSR lines, and heritability of the trait was about $h^2 = 0.26$. Mice from these early generations of selection were tested for other signs of ethanol withdrawal and were found to differ in some e.g., tremor) but not all (e.g., reduced activity) other ethanol withdrawal signs (Kosobud and Crabbe 1986). WSP mice have more severe

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withdrawal HIC after acute or chronic treatment with numerous other sedative hypnotic compounds, and they also differ in a number of other behavioral and neuropharmacological features (for reviews, see Metten and Crabbe 1996; Finn et al. 2004).

Naive mice from selected generations 7–16 were tested for tolerance to the hypothermic effects of ethanol. Ethanol was given for 3 days at 3.5 g/kg ip, and the reduction in body temperature was measured. By the third day, significant chronic tolerance was seen, but there was no significant difference between WSP and WSR lines in the magnitude of tolerance. An addition experiment gave 3.5 g/kg ethanol twice daily for 5 days to increase the amount of tolerance that developed, but WSP and WSR mice still did not differ.

Separate groups of mice were tested for attenuation of the duration of the loss of righting reflex following three daily 4 g/kg injections. Neither selected line developed chronic tolerance. Ethanol was then given twice daily at 3.5 g/kg for three days, and loss of righting reflex duration tested on the fourth day after 4 g/kg (parallel groups received saline only on days 1–3). Both WSP and WSR mice developed significant tolerance, but to an equivalent extent.

In all the above experiments, mice of both replicates of the selected lines were tested, with equivalent outcomes. This greatly strengthens the interpretation of a lack of a genetic correlation between tolerance measures and chronic withdrawal severity, as apparently correlated responses to selection can arise by chance in the relatively small populations of mice maintained in long term selected lines if there is only one pair of selected lines (Crabbe et al. 1990). For the hypothermic tolerance studies, blood ethanol concentration (BEC) assays confirmed that the tolerance was functional. In contrast, the fact that tolerant WSP and WSR mice regained righting reflex at the same BECs as those responding to their first alcohol injection indicated that the tolerance to loss of righting reflex in this experiment was pharmacokinetic.

Several other experiments have been performed to selectively breed mice for withdrawal severity. Some lines were made dependent using a liquid diet (Berta and Wilson 1992; Wilson et al. 1984) and others have used vapor inhalation (see Kosobud and Crabbe 1995). Yet others have been bred for the severity of acute withdrawal HIC (Metten et al. 1998). Unfortunately, none of these lines were ever tested for tolerance to any ethanol response, and all are extinct.

Mouse lines have also been selectively bred for two forms of ethanol tolerance. Starting with a segregating stock, two replicate pairs of mouse lines were selected for high (HAFT) or low (LAFT) acute functional tolerance to ethanol using a dowel balancing task (Erwin and Deitrich 1996). Mice were given an ip injection of 1.75 g/kg ethanol and repeatedly placed on a dowel beginning several minutes after injection until they could remain on the dowel without falling for 30 s. A blood ethanol sample (BEC_1) was taken. They were then injected with a dose of 2.0 g/kg and later tested again until they recovered ability to remain on the dowel. BEC_2 indexed this second recovery point. Acute functional tolerance (AFT) was defined as the difference in BECs (BEC_2-BEC_1).

High (HRT) and low (LRT) rapid tolerance mouse lines (in replicate) were selectively bred from a heterogeneous stock for a different tolerance phenotype (Rustay and Crabbe 2004). Mice were tested for two successive days for the effect of 2.5 g/kg ethanol to impair performance on an accelerating rotarod. The increase in latency to fall (Day 2-Day 1) was the selection index. These animals showed genetic differences in both rapid (two injection days) and chronic (five injection days) tolerance in this task.

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Unfortunately, neither HAFT and LAFT mice nor HRT and LRT mice were ever tested for ethanol withdrawal severity, so the experiments that parallel those performed in WSP and WSR cannot easily be done. HRT and LRT are extinct. HAFT-2 and LAFT-2 mice are preserved cryogenically as embryos, so it would be feasible (albeit expensive) to resuscitate them for the purposes of testing for withdrawal severity.

In summary, the data from lines selectively bred for ethanol withdrawal severity differences suggest that genetic contributions to withdrawal and tolerance phenotypes are generally distinct.

6.2 Standard Inbred Strains

Several data sets have been published documenting mouse inbred strain differences in the severity of alcohol withdrawal. All have employed the HIC to index withdrawal severity. Fifteen inbred strains were studied for 24 h following a single ip injection of 4 g/kg ethanol (Metten and Crabbe 1994). Eighteen strains were exposed to ethanol vapor inhalation for 72 h and found to differ in withdrawal severity (Crabbe et al. 1983). A limitation of this early study was that strains differed markedly in their BEC during inhalation, hence in the dose of ethanol to which they were chronically exposed. We do not consider those data here. This experiment was repeated more recently with 15 inbred strains using a procedure that exposed different strains to different ethanol vapor concentrations in order to match them for experienced dose. This experiment also yielded significant inbred strain differences in withdrawal severity independent of dose administered (Metten and Crabbe 2005). Finally, recent interest has emerged in studying ethanol withdrawal using a procedure where vapor exposure is limited to 16 h/day with 8 h exposure to air, for 3–4 days (Lopez and Becker 2005). Thirteen strains were characterized for withdrawal following this chronic intermittent exposure paradigm (Metten et al. 2010). Two data sets with a substantial number of inbred strains have reported ethanol tolerance magnitude. Eighteen strains were given 3.0 g/kg ethanol ip for 8 days and the reduction in body temperature was assessed at several times following injection on Days 1, 3, 5, and 8. Magnitude of chronic tolerance was indexed as the attenuation of Day 1 hypothermic response on each of Days 3, 5, and 8 (Crabbe et al. 1982). Twenty strains were assessed for the development of acute functional tolerance to the effect of a single dose of ethanol to induce loss of the righting reflex. Acute functional tolerance was indexed as the difference between BEC at regain and loss of righting reflex (Ponomarev and Crabbe 2004).

We examined the strain mean correlations for the three above withdrawal and seven (six hypothermia, one loss of righting reflex) tolerance phenotypes. Depending on strain overlap across studies, these correlations were based on between 8 and 20 strains (see Table 1). Of the 18 correlations between withdrawal and hypothermic tolerance scores, the largest absolute value was $r = 0.34$ ($df = 8$, NS). All correlations with withdrawal severity after chronic continuous exposure were negative, as were two of the six with withdrawal following chronic intermittent exposure. Correlations with acute withdrawal severity were evenly split between positive (after 3–5 days in the hypothermic tolerance regimen) and negative (after 5–8 days) data points. Acute functional tolerance to the loss of righting reflex tended to correlate significantly, and negatively ($r = -0.63$, $df = 7$, $P = 0.07$) with withdrawal from chronic intermittent vapor exposure, but not with acute ($r = -0.31$) or chronic continuous exposure ($r = 0.07$).

These data sets were constructed so that the tolerance measures represent functional tolerance, albeit of two sorts, acute and chronic, and were based on two different behavioral end points. The withdrawal measures also were controlled for ethanol dose experienced. Why was loss of righting reflex tolerance weakly associated with chronic intermittent and not chronic continuous or acute withdrawal phenotypes? This is because these three

withdrawal phenotypes are themselves only imperfectly associated at the genetic level. Overall, the pattern of results suggests that there is no significant degree of overlap in the genetic contributions to withdrawal and tolerance phenotypes.

6.3 Recombinant Inbred Strains

Most recombinant inbred strain data relevant for alcohol genetics have been collected in the BXD RI strain panel. Twenty-one of these strains have been characterized for acute withdrawal severity after a single 4 g/kg ethanol dose (Metten and Belknap, unpublished data). They have also been exposed to continuous vapor inhalation for 72 h and scored for chronic withdrawal severity (Crabbe 1998). No data are available for these strains following chronic intermittent exposure, but these data are currently being collected (H. Becker, personal communication). Twenty-five strains have been tested for hypothermic tolerance to ethanol injections. The grid test was used to characterize the development of tolerance to ambulatory ataxia in 24 strains.

Two different groups assessed functional tolerance to ethanol's effects on a dowel balancing test. One experiment followed the exact procedure employed to breed the HAFT and LAFT selected lines described earlier (Erwin and Deitrich 1996) and found significant RI strain differences in acute functional tolerance (Kirstein et al. 2002). The other study followed a slightly different procedure (Gallaher et al. 1996—see *Sidebar*).

Genetic correlations across withdrawal and tolerance phenotypes measured in the BXD RI strains are shown in Table 2. As seen in the standard inbred strains, chronic hypothermic tolerance was not significantly correlated with any of the three withdrawal measures; nor was chronic tolerance in the grid test. There was, however, a pattern of significant correlation between acute tolerance in the dowel test and chronic withdrawal severity. These correlations accounted for 18 or 34% of the variance, depending on the tolerance assay. While acute withdrawal severity tended to be associated with the tolerance as measured by Gallaher et al. ($r = 0.42$, $df = 19$, $P = 0.06$), it was essentially uncorrelated with tolerance in the Kirstein procedure ($r = 0.06$). As with the standard inbreds, there was, therefore, a lack of complete parallelism of results between tolerance and different withdrawal measures. Again, this was likely because the two dowel test tolerance measures were themselves very weakly associated ($r = 0.23$), and the two withdrawal measures were imperfectly associated ($r = 0.63$).

6.4 Summary of Rodent Data

Data from rodents do not in our opinion offer strong and consistent evidence for a genetic relationship between the various tolerance and withdrawal phenotypes explored. The strongest evidence for such an association was seen in the BXD RI strains, where chronic withdrawal HIC severity after chronic continuous administration of ethanol vapor was significantly genetically correlated with tolerance assessed in two different variants of the dowel test. These two tolerance variants resemble AFT, but neither represents the classic version of this type of tolerance. The importance of this relationship for answering to larger questions in human drinkers should be assessed in the context of several qualifications. First, the shared variance accounted for a relatively small proportion of the total variance (18 or 34%). Second, the relationship was only seen for acute withdrawal severity in one of the two data sets, and even there the correlation was small ($r = 0.42$). Third, the only genetic variance in these data sets arose from alleles polymorphic between C57BL/6J and DBA/2J inbred progenitor strains. Fourth, the only hint of a relationship in standard inbred strains, where there is substantially greater genetic diversity, was a trend toward a negative genetic correlation between AFT to a different behavioral endpoint and one, but not two other, measures of withdrawal severity. Finally, no evidence of consequential differences in

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several measures of tolerance to two different behavioral end points was seen between mice bred to have very large differences in acute and chronic withdrawal severity.

7 Consilience in Studies of Gene Expression

All the rodent studies reviewed above were designed to explore one source of genetic variation, due to allelic differences at genes, i.e., polymorphisms. The allelic differences were either chance occurrences (inbred strains) or engineered by affecting allelic structure through systematic selective breeding. Such genetic differences can be traced to differences in DNA sequence, and such polymorphisms appear in all cells, at all times. Another source of genetic variation is also important. Not all copies of each gene are constantly expressed. Gene expression leading to RNA and protein synthesis clearly differs across time including developmental course, and different genes show very different temporal patterns of expression. The same gene may show very different temporal patterns of expression in different brain areas. To understand genetic influences on AD risk we therefore need to consider brain-regional differences in the expression of genes and how they are affected by chronic ethanol.

As noted in the previous sections, the two rodent phenotypes that can be most closely related to human AD are withdrawal and tolerance. Unfortunately, neither of these phenotypes has been extensively studied from the perspective of global gene expression. In contrast, there are extensive gene expression data on preference drinking (see e.g. Mulligan et al. 2006; Tabakoff et al. 2009; McBride et al. 2010). With this point in mind, the discussion on the expression data has been expanded to include preference drinking, recognizing that this phenotype only imperfectly aligns with any aspect of the AD associated symptoms. Before reviewing the expression data, there are several issues that require comment.

The first issue is that the brain regions and circuits associated with alcohol-related phenotypes are still being defined. A role for the corticotropin releasing factor-rich central nucleus of the amygdala (CeA) in withdrawal and dependence phenotypes has been suggested (see Roberto et al. 2003; Koob and LeMoal 2005; Koob and Volkow 2010). Chen et al. (2008) found that the lateral aspect of the substantia nigra pars reticulata is required to express the acute withdrawal HIC phenotype in mice; withdrawal from chronic ethanol exposure appears to involve a circuit associated with the CeA, the basolateral amygdale (BLA), the dentate gyrus, the CA3 region of the hippocampus, the lateral septum, and the prelimbic cortex (Chen et al. 2009). Withdrawal from chronic intermittent ethanol exposure appears to involve a very similar circuit (Oberbeck and Hitzemann, unpublished observations). To our knowledge, there are no similar studies focusing on the circuits associated with acute or chronic functional tolerance. For preference drinking it is generally assumed that some aspects of the brain's reward pathways are involved (see Koob and Volkow 2010). However, unlike the situation for stimulant drugs of abuse, 6-hydroxydopamine lesions of the nucleus accumbens (NAc) or the ventral tegmental area have been found in some but not all studies to have little effect on ethanol consumption (see e.g. Rassnick et al. 1993; Fahlke et al. 1994; Ikemoto et al. 1997). Moller et al. (1997) found that lesions of the CeA but not the BLA reduced ethanol consumption in rats. Dhaher et al. (2008, 2009) found that lesions of the CeA but not the lateral posterior portion of the bed nucleus of the stria terminalis or the medial shell region of the NAc reduce ethanol consumption in a limited access two-bottle choice paradigm.

The second and related issue is whether the brain regions associated with human AD have strict counterparts in the rodent brain. For example, Koob and Volkow (2010) emphasize the role(s) of prefrontal areas such as the human orbital prefrontal cortex which may have no equivalent in the rodent (see Price 2007). Peters et al. (2009 and references therein) have

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emphasized a rodent circuit associated with drug abuse that involves the ventromedial prefrontal cortex. Key regions are the infralimbic and prelimbic cortex. In the non-human primate and human brain, these regions most closely align with areas 25 and 32 and rostral aspects of the anterior cingulate. To our knowledge, there are no published studies that have attempted a cross-species comparison of global gene expression across “equivalent” cortical brain regions from mouse or rat to man. It may well be possible to align brain regions based on function rather than anatomical features but this needs to be done cautiously.

The third issue involves the microarray technology used to assess global gene expression. Over the past decade, improvements in both microarrays and analytical techniques have made it possible to measure changes in brain gene expression quite accurately; importantly, the cumulative data indicate that most of the changes associated with behavioral phenotypes are actually quite small and in the range of 15 to 30% (see Mulligan et al. 2006; Bice et al. 2006). To some extent, the small changes reflect the fact that the hybridization isotherms for oligonucleotide arrays are frequently not linear due to probe saturation (Pozhitkov et al. 2010). This is true for both rodent and human arrays; thus, small but significant changes in one species may drop below the threshold for detection in another species, especially given that sample sizes are frequently limited. A related problem that makes comparison across species difficult is the effect of single nucleotide polymorphisms (SNPs) on gene expression (e.g. Peirce et al. 2006; Walter et al. 2007, 2009). False positives and negatives in one species can be difficult to align with another. SNP masking is one solution to this problem but this assumes that one knows most of the high frequency SNPs. A fourth problem with microarrays arises from the annotation and summarization issues associated with predefined reporters/probes (Allison et al. 2006). Annotation problems continue, despite continued improvements in sequence information. Thus, caution still must be exercised when comparing data on “gene X” across species. Furthermore, microarray technology provides limited information about alternative splicing, microRNAs and almost no information about other non-coding RNAs. The importance of the non-coding RNAs to the regulation of gene expression and to our understanding of complex traits has been summarized (Lander 2011). In this regard, the emergence of next-generation sequencing and the RNAseq application provides a clear alternative to microarrays for detecting differential gene expression and effectively deals with the problems noted above (see Mardis 2011).

7.1 Human Expression Data from Post Mortem Brain Tissue

The number of human *post-mortem* global gene expression studies is relatively small but there has been remarkable congruence among the studies (see Liu et al. 2006). To our knowledge Lewohl et al. (2000) were the first to use microarrays (cDNA arrays) to examine gene expression in tissue from alcoholics and matched controls (this also appears to be the first use of microarrays for any alcohol-related study). Although the number of samples and the number of genes interrogated were relatively small, these authors noted some marked differences between groups in the cortical expression of myelin-related genes. A follow-up study (Mayfield et al. 2002) using a larger cohort and an improved array confirmed differences in the expression of the myelin-related genes. Genes affected included myelin-associated glycoprotein, apolipoprotein D, glial fibrillary acidic protein and oligodendrocyte-myelin glycoprotein. An additional key finding was that a number of genes involved in protein trafficking were altered in the alcoholic case groups. Members of this group included genes involved in variety of functions such as vesicle docking, synaptogenesis, and synaptic plasticity. Liu et al. (2006) provided additional data from the same laboratory but also integrated the results across several different laboratories (e.g. Flatscher-Bader et al. 2005; Sokolov et al. 2003). Importantly, these authors identified 27 genes that were changed in alcoholics across multiple studies and included in this list were myelin-related genes e.g. proteolipid protein 1. The repeated observation of an effect on

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myelin-related genes aligns with a well-described alcoholic neuropathology (e.g. Harper and Kril 1990).

There appears to be only a single study that has investigated human global gene expression in a non-cortical area. Kryger and Wilce (2010) examined gene expression in the BLA. The sample included ten alcoholics and ten controls obtained from the New South Wales Tissue Resource Centre at the University of Sydney; samples from this same resource were used in some of the studies described above. A large number of genes were found to be differentially expressed; 212 were up-regulated and 560 were down-regulated. It is impossible to summarize all of these findings here; rather, three main findings are emphasized. (a) There was marked reduction for the alcoholics in the expression of oxyreductases, including many genes associated with energy metabolism; these data are consistent with the results of positron emission tomography scans on alcoholics (Volkow et al. 1992). (b) Protein trafficking and vesicle docking genes showed abnormal expression, which aligns with results from other studies (see above). (c) Kryger and Wilce (2010) emphasize some marked changes in glutamate related genes including *GLAST, GLT-1* and *GluR2*. Western blots were used to confirm protein changes in these genes.

In summary, human *post-mortem* studies have detected some marked changes in gene expression between alcoholics and controls. Despite the many potential confounds, there are some consistent themes which can be linked to the known neuropathology. While such studies may relate to the presumed tolerance and withdrawal experience *ante mortem* by the alcoholic subjects, they cannot distinguish between them as potential drivers of the expression differences as compared with controls.

7.2 Rodent Expression Studies

Rodent studies by and large have taken a different approach to the problem. Rather than looking at the effects of chronic ethanol consumption or exposure, the focus has often been on integrating brain gene expression data with gene mapping analyses, i.e., the emphasis is on finding genes which predispose one to excessive ethanol consumption or pronounced withdrawal symptoms. There have been extensive studies using informative genetic populations to identify the genomic location of quantitative trait loci (QTL). QTL mapping studies have isolated the chromosomal loci associated with many alcohol-related traits, and expression analyses have been employed to seek candidate genes for these QTL. This difference in approach, termed "genetical genomics," is an obvious barrier to achieving consilience between the rodent and human data sets as the approaches differ tactically.

The gene encoding multiple PDZ domain protein (MPDZ), which has been identified as an alcoholism-related gene in human genetic association studies, has been found in mice to be a quantitative trait gene associated with acute ethanol withdrawal HIC severity (Shirley et al. 2004). Ethanol tolerance, the other consilience phenotype, has been less well studied. Hu et al. (2008) used the genetical genomic approach to identify eight genes associated with AFT. Interestingly, one of these genes, erythrocyte membrane protein band 4.1-like 2 (*EPB41l2, or 4.1G*) is a cytoskeletal protein that interacts with AMPA receptor GluR1 and GluR4 subunits, and may support their surface expression (Coleman et al. 2003). Bell et al. 2009 examined the effects of chronic ethanol exposure in the alcohol preferring P rats on gene expression in the nucleus accumbens; neither the degree of tolerance that may have developed nor whether dependence had developed were assessed. Two different paradigms were used to administer ethanol chronically: (a) multiple scheduled access (three, 1-hr, dark-cycle sessions/day) for 8 weeks; and (b) continuous, daily alcohol access (24 h/day). The control group was ethanol na. Average daily ethanol intakes for the continuous and multiple groups were approximately 9.5 and 6.5 g/kg/day. Animals were sacrificed 15 h after the last ethanol exposure: assuming that the animals were dependent, the animals were in the early

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stages of withdrawal. Interestingly, the multiple access group showed few changes in gene expression. In contrast, 374 genes were detected as significantly different between the continuous access and ethanol-na groups. Twenty significant Gene Ontology (GO) categories were over-represented and these included negative regulation of protein kinase activity, antiapoptosis, and regulation of G-protein-coupled receptor signaling. Some of the differentially expressed genes were ones that had been detected in human *post mortem* studies, e.g., *Scg2* (Mayfield et al. 2002).

Gene expression profiling has been used to study ethanol withdrawal severity in the WSP and WSR selected lines of mice (Hashimoto and Wiren 2008). Mice of both WSP and both WSR lines were exposed to equal ethanol vapor concentrations for 72 h. Eight hours into withdrawal, when HICs would have been marked in the WSP mice (and virtually absent in the WSR mice), the prefrontal cortex was harvested for microarray analyses. Mice were not scored for withdrawal, however. Mice of both sexes were tested. Ethanol withdrawal clearly regulated the expression of approximately 300 genes. Interestingly, there were large sex differences in the pathways identified by gene ontology overrepresentation analyses. These, and pathological analyses, were consistent with greater ethanol neurotoxicity experienced by female mice. However, there were no important differences between the WSP and WSR mice in the GO categories. Thus, while the genes and gene pathways identified clearly were ethanol withdrawal-responsive, they were not germane to the issue of the large genetic differences in ethanol withdrawal severity between WSP and WSR mice (Hashimoto and Wiren 2008). Another project examined cingulate cortex and amygdala tissue in Wistar rats after a long period of ethanol exposure (Rimondini et al. 2002). Rats were exposed to ethanol vapor for 8 weeks and then drank voluntarily for several weeks. Expression analyses of tissue harvested after recovery revealed several genes and pathways to be chronically up-regulated. This study and several others have been recently reviewed (Bjork et al. 2010).

It should be noted that at least among mice, there is an inverse genetic relationship between ethanol preference and severity of withdrawal HIC (Metten et al. 1998; Hitzemann et al. 2009). Thus, some candidate genes for preference phenotypes, and their associated gene networks, are highly likely to be involved in withdrawal, one of the target phenotypes for this discussion. Sandberg et al. (2000) appear to have been the first to integrate behavioral QTL and gene expression data. Three symposia reports (Hoffman et al. 2003; Matthews et al. 2005; Sikela et al. 2006) illustrate the application of this approach to alcohol related phenotypes, which were mostly based on ethanol preference drinking. Mulligan et al. (2006) performed a meta-analysis using microarray data from six different samples of alcohol preferring and non-preferring animals. The data from a total of 107 arrays were entered into the analysis. The statistical power of the analysis allowed the authors to detect 3,800 genes uniquely and significantly changed between preferring and non-preferring animals. Several functional groups, including mitogen-activated protein kinase signaling and transcription regulation pathways, were found to be significantly over-represented. Focusing on the genes within the mouse chromosome 9 QTL for ethanol preference which has been detected in multiple studies (Belknap and Atkins 2001), several genes were detected as being highly differentially expressed between preferring and non-preferring animals; genes included in the group are *Scn4b, Scd5* and a number of genes with unknown function. *Scn4b*, which reduces ethanol effects on sodium channels, is currently under investigation using transgenic and viral mediated transfer strategies (Hitzemann—unpublished observations).

Tabakoff et al. (2008) used a somewhat different approach to detect genes associated with ethanol preference; however, Scn4b still emerged as a strong candidate. Importantly, this group has consistently found that *Gnb1* is differentially expressed between preferring and non-preferring animals. This group extended this observation and also summarized the genes in mouse, rat and humans that have been associated with excessive ethanol

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consumption (see Table 2 in Saba et al. 2011). These authors concluded that the activity of the GABAergic system, and in particular GABA release and GABA receptor trafficking and signaling including G protein function, contributes significantly to genetic variation in the predisposition to varying levels of alcohol consumption. This conclusion aligns with the known mechanisms of ethanol action (Spanagel 2009).

7.3 An Alternative Strategy for Relating Gene Expression to Function

The human and rodent gene expression studies outlined here all largely used the same data analysis tactic which focused on finding genes that are differentially expressed and then aligning these genes with known protein–protein interaction pathways. There are alternative analysis strategies. For example, the covariance structure of the gene expression data can be analyzed. One such tactic is the weighted gene co-variance network analysis (WGCNA) (Zhang and Horvath 2005). The advantages of this approach over looking at differential expression are discussed in Zhao et al. (2010). Here, we simply note that the focus is on looking at gene connectivity both within and between gene expression modules which may or may not be associated with differential expression. The disadvantage of this approach is the requirement for large sample sizes (see Iancu et al. 2010). Oldham et al. (2006) used WGCNA to examine the conservation and evolution of gene coexpression networks in human and chimpanzee brains. The data obtained illustrated two important points. The first is that in both the human and chimpanzee brains, modules that correspond to brain regions would not be successfully detected simply on the basis of differential gene expression among brain regions. The second point is that the coexpression analysis led to the observation that the "[degree of] conservation of gene coexpression modules between the species recapitulates evolutionary hierarchy, with white matter > cerebellum > caudate nucleus > caudate nucleus + anterior cingulated cortex > cortex, again a relationship not evident from differential expression analysis." In a second study, Oldham et al. (2008) used WGCNA to examine the functional organization of the human brain transcriptome. Here, the coexpression analysis was able to parse the microarray data to identify different modules of coexpressed genes that corresponded to neurons, oligodendrocytes, astrocytes and microglia. Importantly, it was possible to place a number of genes of unknown function into one of these modules. As larger alcohol-related data sets for both human and rodent data evolve, it is not unreasonable to expect that it will be possible to use strategies such as the WGCNA to detect cross-species consilience in the gene expression data.

8 Conclusions and Future Directions

For the only human AD diagnostic criteria for which there are substantial rodent data, alcohol withdrawal and tolerance, those data largely support their genetic independence. Albeit quite limited in extent, nonetheless, we do observe a limited consilience between rodents and humans in this regard—that the genetic risk factors for alcohol-related tolerance and withdrawal are largely uncorrelated. This is true despite the close relationship between tolerance and dependence as pharmacological characteristics of chronic drug administration. That is, it is not likely to find an animal that is dependent but not tolerant.

For humans, many problems ensue in individuals as a result of their heavy drinking including work-related issues, fraying or disintegration of family relationships, psychiatric problems such as anxiety or depression and legal difficulties. Of these, only anxiety-like and depressive-like behaviors are modeled in rodents. Humans continue to drink despite increasing evidence of adverse medical or social consequences—indeed, some alcoholics effectively drink themselves to death. As discussed in Sect. 1, rodents do not drink enough alcohol under most circumstances to develop the sorts of major medical complications seen in humans with AD. However, one approach has recently been suggested to represent drinking despite adverse consequences. When rats or mice have been led to drink

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chronically under certain conditions, subsequent adulteration of the alcohol solution with quinine does not have the expected effect of reducing consumption as strongly as in animals with less experience drinking alcohol. Because quinine is normally aversive, this persistent drinking in the face of a normally punishing event may be considered to reflect, at least partially, drinking despite adverse consequences (Lesscher et al. 2010). However, such studies to date have not shown *why* ethanol-experienced animals fail to avoid ethanol + quinine. Persistent drinking of an adulterated ethanol solution could simply reflect a change in the salience of the taste of quinine; we recently reported a rather direct example of carryover effects of alcohol solutions that abolished the subsequent taste preference for sweet solutions and the avoidance of quinine solutions (Crabbe et al. 2011).

Several approaches may begin to address the “loss of control” over drinking. When alcohol is offered in a standard two-bottle preference test with water as an alternative, it was noted many years ago that following a period of abstinence, ethanol preference drinking is escalated for a time after the re-introduction of access. This has been termed the alcohol deprivation effect, or ADE (Sinclair and Senter 1967). A recent variant of this procedure produces a robust increase in alcohol drinking in C57BL/6J mice (Melendez et al. 2006; Melendez 2011). However, the elevated drinking does not persist for very long, and no blood alcohol levels were taken in these experiments, so whether the animals reach intoxicating blood alcohol levels is not known. And, there are no published data currently available regarding genetic differences in this type of drinking. Scheduling access to fluids for a limited period each day leads C57BL/6J mice to drink large amounts and reach intoxicating blood ethanol levels (Finn et al. 2005; Cronise et al. 2005). A possibly related method is to offer ethanol to animals intermittently, either every other day (Wise 1973) or 3 days\week (Simms et al. 2008), but these animals do not reach intoxicating blood alcohol levels. Cunningham and collaborators have exposed mice to ethanol chronically via indwelling gastric cannulae. Mice thereafter are willing to self-administer ethanol via intragastric infusion, and even a normally alcohol-avoiding genotype, DBA/2J, will self-administer substantial doses of alcohol (Fidler et al. 2011). We have bred high drinking in the dark (HDID) mice for binge-like drinking. These mouse lines drink to the point of behavioral intoxication, but have not been studied for tolerance or withdrawal traits (Crabbe et al. 2009). With the exception of the HDID selection, these phenotypes have yet to be genetically characterized extensively enough to address the questions at hand regarding human diagnostic criteria, but they may provide useful tools for future translational studies.

There are other data that would be very helpful if they were available. The tolerance mouse lines, HAFT and LAFT, now exist only in cryopreserved embryos, but could be resurrected and tested for withdrawal severity. This would offer a rather direct test of the genetic correlation between withdrawal and tolerance. The HRT and LRT mouse lines, selected for rapid (chronic) tolerance, would also be useful, but were not cryopreserved. A thorough characterization of the WSP and WSR selected lines for a range of ethanol tolerance phenotypes would be informative.

The existing data on gene expression differences, either predisposing to alcohol responses or consequent to exposure to alcohol, have unfortunately rarely characterized either tolerance or withdrawal phenotypes. A systematic characterization of the gene expression networks predisposing to and invoked by ethanol tolerance could be compared with the networks seen after initiating dependence, and during withdrawal. A maximally informative line of mice for such a genetic experiment would be the HS/CC outbred stock, which was developed by members of the Collaborative Cross consortium specifically to display maximal allelic diversity (Churchill et al. 2004).

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These results have obvious implication for efforts in human populations to study genetic risk factors for AD. In twin or adoption studies, which assess aggregate risk factors across the genome, prior studies that have looked at the magnitude of genetic effects, developmental processes or patterns of comorbidity have consistently assessed AD as if it reflected a single dimension of genetic liability. These results will need to be reconsidered in light of evidence for multiple genetic factors underlying AD. Molecular genetic studies—particularly candidate gene and genome-wide association studies—have similarly focused almost exclusively on the comparison of subjects meeting criteria for AD with matched controls. If correct, the results reviewed herein suggest that this approach would be at inefficient at best. Cases and controls would likely differ on three relatively independent dimensions of genetic risk with the degree of difference varying considerably across individuals. While these results need replication before they should lead to widespread changes in analytic strategy, they highlight the assumptions widely accepted but rarely tested that psychiatric and substance use disorders as described by current diagnostic systems reflect a single dimension of genetic risk. This assumption is unwarranted and should not be accepted prior to being subject to empirical test.

In summary, further refinements in both the human and rodent laboratory data are needed to determine whether AD represents one or multiple genetic factors. With the growing power of genetic analyses, it should be possible to improve our insight into human etiology, even if it is not possible to resolve completely the specific issue reviewed here.

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Abbreviations

AA/ANA	Alko Alcohol/Nonalcohol rat selected lines
AD	Alcohol dependence
ADH	Alcohol dehydrogenase
AFT	Acute functional tolerance
ALDH	Aldehyde dehydrogenase
BEC	Blood ethanol concentration
BLA	Basolateral amygdala
BXD RI	Recombinant inbred strains derived from crossing C57BL/6J and DBA/2J inbreds
C57BL/6J	A common inbred strain of mice
CA3	Region of hippocampus
CeA	Central nucleus of the amygdala
DBA/2J	A common inbred strain of mice
DSM-IV-R	Diagnostic and Statistical Manual of the American Psychiatric Association
FHP/FHN	Family history positive/negative
GABA	Gamma aminobutyric acid

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GLAST	Gene encoding the glutamate-aspartate transporter
GLT-1	Gene encoding a glutamate transporter
GluR1,2,4	Genes encoding glutamate receptor subunits
Gnb1	Gene encoding the guanine nucleotide binding protein beta 1 subunit
GO	Gene ontology
HAFT/LAFT	High/Low Acute Functional Tolerance mouse selected lines
HAPLAP	High/Low Alcohol Preferring mouse selected lines
HDID	High Drinking in the Dark mouse selected line
HIC	Handling-induced convulsion
HT	Hypothermia
HRT/LRT	High/Low Rapid Tolerance mouse selected lines
NAc	Nucleus accumbens
P/NP	Preferring/Non-preferring rat selected lines
QTL	Quantitative trait locus/loci
Scd5	Gene encoding a stearoyl-CoA desaturase isoform
Scn4b	Gene encoding the sodium channel 4b subunit
SNP	Single nucleotide polymorphism
WDR	Withdrawal
WGCNA	Weighted gene covariance network analysis
WSP/WSR	Withdrawal Seizure-Prone/-Resistant mouse selected lines

A.1 Appendix

Sidebar. Mouse measures of tolerance and withdrawal severity in Tables 1 and 2

Trait	Description	Tabled variables	Reference
Hypothermic tolerance (standard inbred strains)	Mice were injected daily for 8 days with 3.0 g/kg EtOH. Initial hypothermic sensitivity was indexed as difference scores, each representing the reduction (on day 1) from baseline at 30 or 60 min after injection. Tolerance on days 3, 5, and 8 was indexed as the difference in post-injection change score from day 1 sensitivity score	Table 1, traits 1 and 2 tolerance on day 3 (e.g., HT chronic 30–3 and HT chronic 60–3) Table 1, traits 3 and 4, tolerance on day 5 Table 1, traits 5 and 6, tolerance on day 8	Crabbe et al. (1982)
Acute functional tolerance Loss of righting reflex (standard inbred strains)	Mice were injected with 3.0 g/kg EtOH. Blood samples were taken when they lost the righting reflex (i.e., were unable to turn over from a supine position) and when they regained it. The difference in blood EtOH concentrations (recovery minus initial loss) indexed AFT	Table 1, trait 7 AFT LORR	Ponomarev and Crabbe (2004)
Acute withdrawal (standard inbred strains)	Mice were injected with 4.0 g/kg EtOH and the handling-induced convulsion (HIC) was scored before, and hourly after for 12 h. Withdrawal severity was indexed as the area under the HIC curve corrected for baseline HIC	Table 1, trait 8 Acute WDR	Metten and Crabbe (1994)
Chronic withdrawal—continuous	Mice were continuously exposed to EtOH vapor for 72 h at an average blood EtOH concentration of 1.6 mg/ml. Withdrawal HIC severity was	Table 1, trait 9 Chronic Cont. WDR	Metten and Crabbe (2005)

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Trait	Description	Tabled variables	Reference
(standard inbred strains)	assessed hourly for 10 h and again at 24 and 25 h. The average area under the 25 h HIC withdrawal curve for each strain was corrected by subtracting the area for HIC scores from a group exposed to air		
Chronic withdrawal—intermittent (Standard inbred strains)	Mice were exposed to EtOH vapor for 16 h/day for 3 days at an average blood EtOH concentration of 1.7 mg/ml. Withdrawal HIC severity was assessed hourly for 10 h and again at 24 and 25 h. The average area under the 25 h HIC withdrawal curve for each strain was corrected by subtracting the area for HIC scores from a group exposed to air	Table 1, trait 10, Chronic Interm. WDR	Metten et al. (2010)
Hypothermic tolerance (BXD RI recombinant inbred strains)	Mice were injected daily for 3 days with 2.0, 3.0 or 4.0 g/kg EtOH. Initial hypothermic sensitivity was indexed as the average difference from baseline at 30 and 60 min after injection on day 1. Tolerance on days 3, 5, and 8 was indexed as the difference in post-injection change score from day 1 sensitivity scores	Table 2, traits 1–3 HT chronic 2 g/kg, HT chronic 3 g/kg, and HT chronic 4 g/kg	Crabbe et al. (1994, 1996)
Grid test tolerance (BXD RI recombinant inbred strains)	Mice were injected with saline for two days, and EtOH 2.0 g/kg on days 3,5,7,9, and 11. The grid test was used to assess foot fall errors through a wire mesh floor on each EtOH day, corrected for locomotion. Tolerance was indexed as the difference between ataxia ratios (foot falls/ activity) on days 11 and 3	Table 2, trait 4 Grid test	Phillips et al. (1996)
Acute functional tolerance Dowel test (BXD RI recombinant inbred strains)	Mice were injected with 1.75 g/kg EtOH and placed on a stationary, 1.27 cm dowel, from which they soon fell. A blood sample was collected when they recovered ability to stay on the dowel (BEC1), and they were given a second, 2.0 g/kg injection. Another blood sample (BEC2) was taken when they again regained ability. AFT was indexed as BEC2 minus BEC1	Table 2, trait 5 AFT Dowel (Kirstein)	Kirstein et al. (2002)
Acute functional tolerance Dowel test (BXD RI recombinant inbred strains)	Mice were given an injection of 2.0 g/kg EtOH. Brain EtOH levels were taken within 10 s of fall from a rotating, 5 cm dowel to assess initial sensitivity. Separate groups of mice were given the initial 2.0 g/kg injection, and when they recovered ability to stay on the dowel, a blood sample was taken. Mice were then given a "booster" dose of 1.0 g/kg and a second recovery was assessed. Four to five booster doses were given until each RI strain of mice was recovering function at a stable plateau of blood EtOH concentrations. The difference between the final and the initial brain EtOH concentration was taken as the index of tolerance	Table 2, trait 6 AFT Dowel (Gallaher)	Gallaher et al. (1996)
Acute withdrawal (BXD RI recombinant inbred strains)	Same as for standard inbreds	Table 2, trait 7 Acute WDR	P. Metten and J.K. Belknap, unpublished data, with permission
Chronic withdrawal—continuous (BXD RI recombinant inbred strains)	Same as for standard inbreds. Average blood EtOH concentration was 1.5 mg/ml	Table 2, trait 8 Chronic cont. WDR	Crabbe (1998)

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Table 1

Inbred strain correlations between alcohol tolerance and withdrawal phenotypes

	Trait	2	3	4	5	6	7	8	9	10
1	HT chronic 30-3	**0.85**	**0.91**	**0.49**	**0.71**	**0.66**	−0.16	0.10	−0.09	0.34
2	HT chronic 60-3		**0.74**	**0.56**	**0.63**	**0.75**	−0.23	0.02	−0.09	0.32
3	HT chronic 30-5			**0.63**	**0.89**	**0.83**	−0.04	0.12	−0.06	0.25
4	HT chronic 60-5				**0.52**	**0.60**	−0.12	−0.20	−0.05	0.16
5	HT chronic 30-8					**0.92**	0.18	−0.19	−0.25	−0.05
6	HT chronic 60-8						0.05	−0.23	−0.24	−0.06
7	AFT LORR							−0.31	0.07	**(−0.63)**
8	Acute WDR								**0.57**	0.38
9	Chronic cont. WDR									**0.59**
10	Chronic interm. WDR									

Correlations in bold, $P < 0.05$ ($P = 0.07$). Correlations are based on 8–20 strain means

Variables 1–6 are from Crabbe et al. (1982). "HT chronic" = hypothermic tolerance, indexed as change from baseline temperature. "30–3" indicates 30 min after baseline temperature on the 3rd injection day, "60–8" refers to 60 min after baseline on the 8th injection day, etc

"AFT-LORR" is the acute functional tolerance to the loss of righting reflex from Ponomarev and Crabbe (2004)

"Acute WDR" is the area under the withdrawal handling-induced convulsion curve after a single alcohol injection from Metten and Crabbe (1994)

"Chronic cont. WDR" is the area under the curve for HIC following 72 h continuous vapor inhalation from Metten and Crabbe (2005)

"Chronic interm. WDR" is the area under the HIC withdrawal curve following intermittent vapor exposure from Metten et al. (2010)

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Table 2

BXD Recombinant Inbred strain correlations between alcohol tolerance and withdrawal phenotypes

	Trait	2	3	4	5	6	7	8
1	HT chronic 2 g/kg	−0.13	0.22	0.05	−0.31	0.31	0.23	0.11
2	HT chronic 3 g/kg		0.13	−0.26	0.14	−0.02	0.06	0.20
3	HT chronic 4 g/kg			−0.20	−0.03	−0.01	−0.08	0.13
4	Grid test				0.20	0.17	0.24	0.10
5	AFT Dowel (Kirstein)					0.23	0.06	**0.43**
6	AFT Dowel (Gallaher)						**(0.42)**	**0.58**
7	Acute WDR							**0.63**
8	Chronic cont. WDR							

Correlations in bold, $P < 0.05$ ($P = 0.06$). Correlations are based on 8–32 strain means

“HT Chronic” variables refer to the attenuation of the hypothermic response to the stated dose of ethanol after three daily injections, as described in Crabbe et al. (1994, 1996)

“Grid test” data are locomotor ataxia scores from Phillips et al. (1996)

“AFT Dowel” are the acute functional tolerance scores on the dowel test, using two different methods (Kirstein et al. 2002; Gallaher et al. 1996)

“Acute WDR” are handling-induced convulsion scores as described for Table 1. Data are from unpublished studies conducted by P. Metten and J. Belknap, with permission

“Chronic Cont. WDR” are as described in Table 1, from Crabbe 1998