Rodent Models of Genetic Contributions to Motivation to Abuse Alcohol

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Introduction

The distinction between alcohol use, which is normative, and abuse, which is unfortunately common, implies dysregulation of motivation directed toward the drug. Genetic contributions to individual differences in the patterns and degree of human alcohol use and abuse account for about half of population variability (Goldman and Ducci 2007). Genetic pathways to abuse risk are mediated through personality differences and other predispositions to drink excessively as well as through differences in sensitivity to the acute and chronic consequences of the drug. Disentangling risk factors from consequences is difficult in human studies, which are usually not prospective: Subjects are most often ascertained based upon a diagnosis of alcohol dependence or a family history thereof. Both risk factors for and consequences of alcohol abuse can be modeled with reasonable fidelity in laboratory rodents. Rat and mouse studies offer immense power to characterize genetic contributions to individual differences, as well as to manipulate the genome of the animals. Specific genes may be targeted for over- or underexpression of their effector proteins. This chapter discusses the efforts to address the motivational aspects of individual differences surrounding alcohol use and abuse in rodent genetic animal models.

Not surprisingly, the biggest challenge remains relating human motivation (which can not only be inferred but also self-reported) in a convincing way to the underlying motivation for rodent behavior directed toward and resulting from alcohol. Despite the intrinsic difficulty, substantial progress has been made (Crabbe 2012), and new approaches are appearing.
A Little History

The willingness of laboratory rats to drink alcohol solutions was reported as early as 1926 by Curt Richter (Richter 1926) who went on to develop what we now term the “two-bottle preference test” to determine taste thresholds for various substances (e.g., salt, sugar, etc.). He reported that when ethanol was offered by choice versus tap water, his rats apparently could not detect the taste of alcohol at concentrations below 1.8%. This was inferred from preference ratios (amount of alcohol ingested/total fluid ingested) that were approximately 50%. They preferred concentrations up to and including 4.8% over water, but rejected concentrations greater than 6% (Richter and Campbell 1940). Data that there were genetic differences as well as individual differences in “alcohol appetite” were reported for both rat and mouse strains where the animals were also offered various diets. Interestingly, this study reported a gene X environment interaction, as “strain O” rats drank more alcohol than “strain H” rats when on diet “A,” but less than “strain H” rats when both were consuming diet “B” (Williams et al. 1949). Exactly what these genotypes actually were is anyone’s guess, as the now-standard genotypes were in considerable flux (although Williams may have used some early version of “dba” and “C3H” mice).

In 1948, Jorge Mardones began to create a rat line that preferred to drink 10% ethanol versus water and named it the University of Chile B (UChB) line; he also bred a UChA line for low ethanol preference (Mardones 1951, 1960). In selective breeding, mating animals with extreme values on the selected trait have the effect of capturing the allelic forms of the genes that influence that trait in the line. In each generation, their offspring show further enhancement of their genetic predispositions until all genetic variation affecting the trait (e.g., high preference drinking) has been exhausted. Comparisons of high versus low selected lines then can reveal other differences in neurobiology and behavior between the lines, which are logically attributed to the influences of the same genes affecting the selected response.

Thus, before 1950, two of the principal approaches to the study of the genetics of preference drinking were already established—studies of natural strain variation and creation of a genetically modified rodent. I will return to the studies of selectively bred lines in a later section. Gene targeting (knockouts, transgenics) was not developed until 1988. The ability to turn on or off the function of individual genes was first used to study alcohol responses in 1996. Many genes have since been targeted and studied for alcohol-related responses: About 80% of those mutants have been assessed for preference drinking. A systematic review of such studies appeared in 2003 (Cunningham and Phillips 2003). Animals targeted for about 25 genes had been tested, and for one third, preference drinking significantly increased. Another one third showed significantly reduced drinking, and the remainder had no significant effect on preference. A subsequent survey of 75 genes showed the same 1/3-1/3-1/3 pattern of outcomes (Crabbe et al. 2006). I stopped tracking new knockout/transgenic studies in 2008, by which time 86 genes still showed the same division of outcomes. Subsequent gene targeting studies have been compiled, and many more effects on preference drinking are documented in a more recent review.
Rodent Models of Genetic Contributions to Motivation to Abuse Alcohol

(Bilbao 2013). These organisms are extremely powerful tools, but a review of all the individual gene effects in the alcohol research literature is beyond the scope of this chapter, as is consideration of the methods (e.g., tissue-specific, conditional knockouts).

The two-bottle preference test has proven to be extremely useful for genetic studies to assess motivation for alcohol (Crabbe et al. 2010). It is very easy to implement and can be conducted with high experimental throughput. We have an historical base of 60 years of rat data and 50 of mouse. Consistent with the fidelity of genetic differences (see below), the heritability of alcohol preference is fairly substantial. Nonetheless, for assessing motivation for ethanol, this behavioral assay also has some limitations. It may be that an animal with a high alcohol preference score is experiencing more reward from ingesting alcohol than an animal with a low preference score. Alternatively, it may be experiencing less and is therefore increasing its intake in an effort to achieve greater reward. Low-preferring genotypes may be sensitive to alcohol’s aversive effects. Another limitation is that although using this test as a screen for potential drug therapies is easily implemented, such studies have not been successful in discriminating therapeutically effective compounds from those that do not perform in human studies. This is largely because there are a great number of false-positive findings in the animal studies (Egli 2005). As will be discussed below, few animals, even from highly preferring genotypes, are willing to ingest sufficient ethanol to become intoxicated (Crabbe 2012). Nonetheless, the test remains by far the most common assay used to assess motivation for alcohol in rodents.

Recognizing the advantages of the mouse over other mammals for genetic studies (e.g., relatively small size and rapid generation time, the availability of many well-defined and replicable genotypes), Gerald McClearn was one of the pioneers of systematic studies of mouse inbred strains. Inbred strains are created by systematic brother–sister matings. Because each offspring inherits half its genetic composition (i.e., one of the two alleles it possesses for each gene) from each parent, half of each individual’s genetic variability is shared by siblings. Thus, genetic variability is halved with each successive generation of sibling matings. After 20 generations, all same-sex mice of a given inbred strain are essentially genetic clones (Falconer and Mackay 1996). Therefore, if one examines a trait in a number of mice from a number of strains and takes care to provide the same environmental conditions to all subjects, the individual differences in the trait can be partitioned into among-strain means (i.e., genetic) and within-strain (nongenetic or environmental) sources. Strain mean differences that significantly exceed pooled within-strain variability in a simple analysis of variance are evidence of genetic influences on the trait.

At the 1968 Nebraska Symposium on Motivation (the year I entered graduate school), Professor McClearn presented a paper entitled “Genetics and Motivation of the Mouse” (McClearn 1968). I consider this a tour de force: It is a paper I have returned to many times, usually to discover that my next, most interesting planned experiment had already been done! After explaining the process of inbreeding, he reviewed the state of the art with inbred strains regarding nearly the entire breadth of mouse behavior. He showed evidence of strain differences in aggression and
dominance; sexual behavior; learning; hoarding and other responses to food; and activity. It is remarkable that he could cover this range in the first ten pages of his manuscript—behavior genetics was a very new field. The bulk of his paper, however, dealt with the topic of this year’s Nebraska Symposium—alcohol studies, specifically the motivation to ingest alcohol solutions. McClearn in 1959 had shown that five inbred mouse strains differed significantly in their preference for 10% ethanol versus water (McClearn and Rodgers 1959). Figure 1 from that paper is reproduced in an adapted form here because it illustrates several findings that have been replicated many times, but are often overlooked. The figure showed data for individual mice from multiple strains, across days of two-bottle preference testing for 10% alcohol versus water.

First, the strain differences are striking. C57BL mice showed nearly total alcohol preference, while DBA mice virtually completely avoided alcohol. Of the other three strains tested, A and BALB/c also avoided 10% alcohol, but C3H/2 showed, on average, intermediate preference scores. Second, for both strains that preferred alcohol, their preference grew over time. Third, there were fairly substantial individual differences within the preferring strains. One of the four C57 mice had only moderate preference, while the other three had nearly total preference. These differences between animals of exactly the same genotype must derive from non-genetic sources. A corollary of this point is that average scores for a genotype may be misleading. Two of the C3H mice had preference ratios of about 30–40% while for the other two the preference ratios were 10% or less. No animals of this strain actually scored very near the mean for the strain. Finally, among the other factors that influence tube choice, the roles of learning and perseverative behavior must be considered. Learning is inferred from the gradual development of preference across days of testing. The hatched vertical lines on days 6–7 and 10–11 in the figure indicate when the position of the tube containing alcohol was reversed with that of the water tube. For all preferring animals, this resulted in a drop in preference scores for at least the following day, followed by recovery to a higher preference. Any investigator who has conducted preference studies knows that there are some animals that exhibit apparent, strong preference for drinking out of the tube on one side or the other of its cage, seemingly oblivious to its content. Fortunately, these are usually relatively rare, and with repeated position switches, the animals tend to find a characteristic preference ratio.

This experiment has been extended to survey multiple inbred mouse strains and has been performed in many laboratories since then: The results of these replications are quite striking. A comparison of multiple-strain data from four laboratories (15–28 strains per study) is shown in Fig. 2. Pairwise comparisons of data from surveys conducted between 1966 and 2006 yielded strain mean correlations ranging from 0.74 to 0.98, indicating the remarkable stability of strain differences in alcohol preference. These correlations reflected stability similar to that from five surveys of strain brain weight conducted between 1967 and 2000 (Wahlsten et al. 2006). While female mice are well known to drink more alcohol than males, Rodgers’ study (included in the historical comparison in Fig. 2) tested both sexes from 19 strains and found that 97% of the variance in preference was accounted for by strain, with only
Fig. 1 Daily preference ratio of consumed 10% alcohol solution to total fluid consumption. Data for four individual male mice per genotype are shown. *Broken vertical lines* between days 6–7 and 10–11 indicate reversal of position of alcohol and water solutions. (Adapted from Fig. 1 in McClearn and Rodgers 1959, with permission)
3% attributable to combined effects of sex, litter, and measurement error (Rodgers 1966). In a later preference study of 22 inbred strains of both sexes offered three different alcohol concentrations serially, we found somewhat different results. There were overall strong main effects of strain and sex (favoring females), but there were also significant interactions of all types. The magnitude of sex differences ranged from marked to none across different strain-concentration combinations (Yoneyama et al. 2008; n.b., it is these data, for 10% ethanol, pooled across males and females for each strain that are included in the Wahlsten comparison and identified as “Finn Lab” in Fig. 2).
McClearn then discussed several other experiments (McClearn 1968). Many of those experiments were designed to assess possible motivations for alcohol ingestion. He established taste thresholds for alcohol by offering multiple solutions of differing concentrations. He found that avoiding strains largely avoided all detectable concentrations, even when as low as 0.05% in the case of the DBA strain. Because this is far lower than could produce any discernible pharmacological effects, this suggests that avoidance in most such strains is strongly influenced by taste or odor, not necessarily by response to alcohol as a drug. He reported that adding sucrose to the ethanol solutions increased consumption, but did not appreciably affect the pattern of strain differences, which is consistent with taste avoidance. When we later conducted studies comparing the ethanol intake of 15 inbred strains for 3 concentrations of alcohol with or without the addition of saccharin, we found a somewhat different result. We found that some strains avoided higher concentrations of alcohol regardless of saccharin’s presence. Other strains showed appreciably higher ethanol intake in saccharin, which we attributed to saccharin’s ability to mask the odor or taste of alcohol. Finally, the remaining strains tended to avoid only the higher concentration of alcohol in saccharin but not water. Since they were ingesting much greater alcohol doses in saccharin, we concluded that they were avoiding the perceived pharmacological effects of alcohol (Belknap et al. 1993). Our subsequent study extended Belknap’s findings to 22 strains and generally supported the earlier conclusions (Yoneyama et al. 2008).

Animals could be consuming alcohol for the calories it provides (Mardones 1951; Richter 1953). McClearn reported that establishing a state of food deprivation did not increase preference in avoiding strains. Forcing food-deprived mice to ingest alcohol solutions allowed mice to maintain body weight more effectively, indicating that alcohol could be used as food. However, the pattern of most and least responsive strains was unrelated to their free-choice alcohol preference (McClearn 1968). In a series of complex experiments serially offering C57BL mice choice between water and different concentrations of alcohol, McClearn tested several hypotheses about how preference might be related to the concentration offered. All concentrations exceeded the taste threshold for this strain. A simplified summary of the outcomes of these experiments is that the amount (i.e., dose) of alcohol ingested increases monotonically and approximately logarithmically with the concentration offered. Thus, across 3, 6, and 12% concentrations of ethanol, C57BL/6 mice showed twice-doubling intakes (McClearn 1968). However, preference ratios show an inverted U function across increasing concentrations, with the peak preference ratio occurring at strain-specific concentrations. This is one reason we advocate reporting consumption (g EtOH/kg body weight) rather than preference ratios in preference experiments. Examination of our two later studies shows increases in dose ingested by C57BL/6 mice in both studies roughly consistent with McClearn’s data, but for many other strains, increases were less or even nonexistent for the highest concentration we studied, 10% (Belknap et al. 1993; Yoneyama et al. 2008). Thus, it cannot be concluded that inbred strains titrate a preferred dose of self-administered alcohol.
McClearn concluded with reports on several early studies attempting to relate rate of alcohol metabolism by alcohol dehydrogenase (ADH) and then aldehyde dehydrogenase (ALDH) to preference differences, mostly between the C57BL/6 and DBA/2 inbred strains. Because these and subsequent studies have failed to yield a consistent relationship between alcohol elimination and preference, they are not discussed here. It is certainly true, however, that alcohol metabolism is an important determinant of risk for alcohol abuse and dependence in humans. A high proportion of humans from East Asian gene pools (e.g., Han Chinese) possess a variant form of the ALDH gene that leads to slow elimination of acetaldehyde after drinking alcohol. Acetaldehyde produces several unpleasant effects including facial flushing, nausea, dizziness, and headaches. Individuals with the slow ALDH variant are at greatly reduced risk of developing alcohol dependence; polymorphisms in ADH also have protective effects, but they are much less marked (Goldman et al. 2005). These allelic variants have much more modest effects in Caucasian/European populations, mostly because they are much more rare in these populations (Liu et al. 2011). This strong genetic effect is a poster child for pharmacogenetics studies hoping to discover novel medications. One of the three therapeutic drugs for alcoholism approved for use in the USA is Antabuse® (disulfiram). Its mechanism of action is to inhibit ALDH activity, and individuals taking Antabuse experience the effects of acetaldehyde if they drink. Unfortunately, its use is limited by the fact that many alcoholics in treatment never are prescribed any of the three effective medications (Saxon and McCarty 2005), and obtaining and monitoring compliance with addiction therapies remains a challenge (Barth and Malcolm 2010). Nonetheless, brain acetaldehyde may play a role in individual differences in alcohol preference in rodents (Socaransky et al. 1984). Recent studies have infused lentiviral vectors into the ventral tegmental area to manipulate brain acetaldehyde levels bidirectionally in high-prefering UChB rats and report modulation of voluntary consumption of ethanol solutions (Karahanian et al. 2011). Adenoviral vectors administered intravenously to alcohol-dependent UChB rats and designed to enhance ADH activity and inhibit ALDH activity led to 60% reductions in drinking (Rivera-Meza et al. 2012). Thus, the animal models may prove useful in discovering adaptations to the rather blunt tool offered by Antabuse for future treatments to alleviate alcoholic drinking.

Indices of Motivation for Alcohol

Assessing motivation in humans is possible by self-report, though not without challenges (e.g., lying or self-delusion). How to assess motivation in laboratory animals is not straightforward, either theoretically or practically (Brown 1961). Because rodents cannot speak to us about their motivational states, we must operationalize their motivation. I have discussed thus far only one such surrogate measure, their tendency to ingest alcohol when water is freely available, and among the earliest studies, nearly all employed this or some closely related assay. An excellent review in the 1968 Nebraska Symposium volume reviewed still new data surrounding
the physiological correlates of both specific (e.g., certain hypothalamic nuclei) and nonspecific (e.g., reticular activation) drive states thought to accompany motivated behavior (Grossman 1968). It is beyond the scope of this chapter to consider the theoretical aspects of motivation in depth. With respect to the dysregulated states associated with addictions including alcoholism, these include both postulated homeostatic disturbances and revaluations of alcohol’s rewarding value, and thus invoke both negative and positive reinforcement to explain behavioral changes. A good example is Koob’s allostatic dysregulation conceptualization, which postulates first an overvaluation of positively reinforcing effects of alcohol leading to regular overindulgence. That increase in chronic excess drinking produces consequences that result in a lowering of a homeostatic set point, particularly with regard to mood and anxiety. Attempts to reestablish the original set point represent a negative reinforcement process termed “allostatic load” that sustains the deviant behavior (Koob 2013). Koob’s work (along with that of many others) strongly implicates neural stress pathways in drug dependence (George et al. 2012). (Interestingly, the same Nebraska Symposium volume also contained a review of then very new data linking stress hormones and behavioral conditioning and extinction (Levine 1968)). While there are numerous other theoretical frameworks for alcoholism from which to choose, all incorporate the notion of dysregulated motivation to consume alcohol (e.g., Sommer and Spanagel 2013; Stephens et al. 2010; West 2006; Blane and Leonard 1999).

In addition to drinking, animals can manifest alcohol-directed behavior by performing work on operant schedules to gain access (Samson and Czachowski 2002). One difficulty with standard operant schedules is that consumption of alcohol is allowed while, for example, animals are working on a progressive ratio schedule to gain additional access. This makes it difficult to distinguish appetitive (drug seeking) behavior from consummatory behavior, as well as having the problem of allowing potential pharmacological effects of the drug to affect responding later in the session. This problem has been circumvented by the use of a progressive ratio schedule which allows only a single reinforcing access to alcohol for each ratio (Samson et al. 2004). Pavlovian conditioning principles have also been used to assess the reinforcing value of an alcohol-induced interoceptive state. Mildly water-deprived rodents will generally learn to avoid a novel taste when administered with intraperitoneal doses of psychoactive drugs including alcohol following each access period. Alcohol-conditioned taste aversion studies have shown reliable strain differences in both inbred mouse and rat strains, as well as differential sensitivity in comparisons of some selectively bred lines. Nearly all of these studies have been reviewed elsewhere, along with taste conditioning studies using other drugs. This chapter also describes studies comparing targeted mutants with controls for sensitivity to an alcohol-conditioned taste aversion; it also lucidly delineates methodological considerations that can influence interpretation of genetic differences in taste conditioning studies (Cunningham et al. 2009). When alcohol injections are paired with exposure to one set of distinctive environmental cues and saline injections are paired with another set of cues, subsequent drug-free tests where both sets of cues are available often reveal a conditioned place preference for the alcohol-
paired side. As with taste conditioning, genetic contributions to individual differences are pronounced and nearly all such studies (including those with mutants) have been reviewed (Cunningham and Phillips 2003).

Methods other than preference drinking can also be used to assess self-administration of alcohol. For most drugs of abuse, the intravenous (iv) route of administration is feasible for rats, and is also used with some success in mice even though technically much more challenging. Alcohol is peculiar in that effective doses are in the gram per kilogram range rather than milligram per kilogram or microgram per kilogram, so large volumes of dilute alcohol solutions would need to be administered i.v., and high concentrations are apparently irritating. Nonetheless, alcohol is occasionally studied by i.v. self-administration. Interestingly, both C57BL/6J and DBA/2J mice will readily self-administer alcohol i.v. (Grahame and Cunningham 1997). Genetic studies with the i.v. route have been reviewed (Green and Grahame 2008). After a state of physical dependence on alcohol is established, rats and mice will also administer alcohol by intragastric infusion (Fidler et al. 2006, 2012) and mouse strains differ in their patterns of self-administration (Fidler et al. 2011). Finally, mice show a locomotor stimulant response to low-to-moderate doses of alcohol similar to that elicited by many other abused drugs. Because the locomotor response, and its tendency to grow with repeated drug administrations (called sensitization), is thought to model the euphoria experienced by drug users (Wise and Bozarth 1987), sensitization to psychomotor stimulant drugs such as amphetamine and cocaine has been widely studied. Alcohol stimulation and sensitization have also shown marked influences of genetics on their magnitude (for review, see Phillips 1997).

Thus, there are genetic differences apparent using any of several different methods to assess motivation for alcohol. There are abundant data for two-bottle preference, but generally sparse genetic data for the other available methods. Are these different rodent behavioral assays assessing the same trait? If so, genotypes showing enhanced motivation for alcohol with one assay should also do so in another. A thorough and thoughtful review of the genetic data concluded that two-bottle preference and operant oral self-administration studies yielded congruent findings (Green and Grahame 2008). This conclusion was largely based on comparisons of the rat lines selected for high versus low preference; high preferrers also tended to show greater operant self-administration. Detailed comparisons of several pairs of these rat lines under different operant conditions not only agreed that there appeared to be some genetic overlap between home-cage drinking and operant oral self-administration but also pointed out that substantial differences in the patterns of self-administration suggested genetic divergence as well (Files et al. 1998; Samson et al. 1998). Genotypes sensitive to an alcohol-conditioned taste aversion were less likely to self-administer alcohol than genotypes with weak conditioned taste aversion. This is a robust finding, as it was seen to distinguish the high alcohol (HAP)- and low alcohol-preferring (LAP) selected mouse lines (Chester et al. 2003; Grahame et al. 2001) as well as in some rat selected lines and, importantly, across multiple inbred mouse strains. These latter genotypes were not selected for either trait, but high preferrers (Belknap et al. 1993) showed low sensitivity to an ethanol-conditioned
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