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Ethanol Drinking in Rodents:

Is Free-Choice Drinking Related to the Reinforcing Effects of Ethanol?

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Abstract

Many studies have used voluntary ethanol consumption by animals to assess the influence of genetic and environmental manipulations on ethanol drinking. However, the relationship between home cage ethanol consumption and more formal assessments of ethanol-reinforced behavior using operant and instrumental conditioning procedures is not always clear. The present review attempted to evaluate whether there are consistent correlations between mouse and rat home cage ethanol drinking on the one hand, and either operant oral self-administration (OSA), conditioned taste aversion (CTA) or conditioned place preference (CPP) with ethanol on the other. We also review literature on intravenous ethanol self-administration (IVSA). To collect data, we evaluated a range of genetic manipulations that can change both genes and ethanol drinking behavior including selective breeding, transgenic and knock-out models, and inbred and recombinant inbred strain panels. For a genetic model to be included in the analysis, there had to be published data resulting in differences on home cage drinking and data for at least one of the other behavioral measures. A consistent, positive correlation was observed between ethanol drinking and OSA, suggesting that instrumental behavior is closely genetically related to consummatory and ingestive behavior directed at ethanol. A negative correlation was observed between CTA and drinking, suggesting that ethanol's aversive actions may limit oral consumption of ethanol. A more modest, positive relationship was observed between drinking and CPP, and there were not enough studies available to determine a relationship with IVSA. That some consistent outcomes were observed between widely disparate behavioral procedures and genetic populations may increase confidence in the validity of findings from these assays. These findings may also have important implications when researchers decide which phenotypes to use in measuring alcohol-reward relevant behaviors in novel animal models.

Keywords

behavioral genetics; instrumental conditioning; classical conditioning; alcoholism; C57BL/6; DBA/2

Introduction

Ethanol is thought to be one of the mostly widely used drugs in the world today. While most ethanol users can be characterized as casual drinkers, the abuse rate is substantial. Alcoholism is a complex psychiatric disorder with an estimated heritability of 50-60%. It has a fairly

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common prevalence world-wide, with the United States showing a prevalence of ethanol dependence as high as 20% in men and 8% in women (Enoch, 2003).

Genetic vulnerability to alcoholism is theorized to be due to multiple interacting genetic loci, each with a small to modest effect combining under certain environmental influences to contribute to vulnerability to ethanol dependence. Animal models such as selectively bred rodent lines as well as inbred and recombinant inbred strains can be used to address this hypothesis of genetic vulnerability. Other animal model literature utilizes knockout and transgenic models in order to focus on single gene alterations to address the specific pharmacogenetics of ethanol use and vulnerability to alcoholism. All of these animal models have been used to study various ethanol-related behaviors, including ethanol drinking. These animal models may lack many aspects of human alcoholism, but experimenters are able to control their genetic and environmental history to research scientific theories difficult to address in human studies due to logistical and ethical considerations. Furthermore, these genetically-based animal models may reveal behavioral, genetic, and physiological characteristics that demonstrate genetic links to behaviors such as ethanol drinking.

Differences in free-choice ethanol consumption have frequently been used to study the genetic and neurobiological mechanisms underlying high ethanol drinking behavior, whether the animal model was created using selective breeding, inbreeding, or targeted gene alteration (Crabbe et al., 1992; Li et al., 1993). One question often arising in the interpretation of these studies is whether high-drinking lines show greater ethanol-reinforced behavior than low-drinking lines. In other words, high drinking is implicitly assumed by many to be evidence of high ethanol reinforcement *per se*. However, other intervening variables such as anxiety (Pohorecky, 1991) or novelty-seeking (Cloninger et al., 1988) have also been speculated to be related to excessive drinking. Furthermore, other variables, such as avoidance of ethanol taste, may interfere with oral measures of ethanol reinforcement. For example, DBA/2J mice drink so little alcohol that they likely never encounter its pharmacological effects (Belknap et al., 1978), they freely self-administer ethanol when it is delivered intravenously (Grahame and Cunningham, 1997). Therefore, it is an open question as to whether animal studies support the idea that genetic differences in free-choice drinking are correlated with genetic differences in more formal assessments of ethanol-reinforced behavior, including those utilizing operant and classical conditioning. The purpose of the present review is to address whether there is, in fact, a consistent relationship between ethanol drinking and behaviors seen when using other methodologies for assessing the reinforcing properties of ethanol.

No matter what method is used to manipulate genes - breeding strategies such as phenotypic selection and inbreeding, or targeted gene alterations using transgenic or knockout techniques - genetic correlations between drinking and other behaviors can be determined. For example, when a pair of selected lines is found to differ significantly on some trait other than the one for which they were selected, one may say that a genetic correlation between the traits exists. Taking this one step further, there may be a common set of genes or gene for the two responses (Crabbe et al., 1990). Such assessment can be useful for eventually understanding underlying mechanisms of action in complex behaviors such as ethanol abuse.

In this review, we will cast as wide a net as possible over different methods for inducing genetic differences in order to be able to detect consistent relationships among differing behavioral phenotypes relevant to ethanol reward and reinforcement. With the non-traditional addition of transgenic and knockout models, one has better power to detect genetic relationships among behaviors because the ability to detect genetic correlations is directly related to the number of genetically varying populations studied (Crabbe et al., 1990).

Oral ethanol self-administration has long been regarded as an index of the reinforcing efficacy of ethanol (e.g., (Myers et al., 1972). Nonetheless, there are other experimental tasks available to address the reinforcing and aversive properties of ethanol. There are two main approaches: Pavlovian and operant conditioning. Pavlovian conditioning includes conditioned place preference (CPP) and conditioned taste aversion (CTA). In these procedures, an association is established between an environmental stimulus, referred to as the conditioned stimulus (CS), and a drug unconditioned stimulus (US). In CTA, the CS is a flavor solution that the subject may consume prior to administration of the drug US, while in CPP, the CS is an environmental cue paired with the effects of the drug US. CPP is frequently used to index the reinforcing properties of self-administered drugs (Tzschentke, 1998). CPP involves pairing a distinct environment with the pharmacological effect of a drug, and a different environment with the absence of the drug. Later the animal is tested in the absence of the drug and freely allowed to occupy or exit the drug-paired environment. Unlike oral self-administration procedures, these tasks allow the ethanol exposure and dose to be controlled by the experimenter. This is a distinct advantage when assessing the rewarding actions of ethanol in genotypes (such as an alcohol non-preferring (NP) rat or DBA/2J mouse) that would not encounter pharmacologically relevant doses of ethanol in a self-administration procedure. Typically, drugs abused by humans cause animals to prefer the drug-paired environment. An important exception is ethanol in rats, in which conditioned place aversion is the typical result. On the other hand, most self-administered drugs produce a conditioned taste aversion, though this behavior has been hypothesized to be positively correlated with sensitivity to drug reinforcement (Hunt and Amit, 1987). These tasks can also be less time-consuming than free-choice drinking experiments (see Chester and Cunningham, 2002 for review).

The other common method of studying reinforcement is to use operant behavioral procedures. Here, a learned instrumental action is required to produce a certain outcome, and the dependent variable is typically the amount consumed, or frequency of behavior directed at the desired outcome. Operant procedures can be used to assess reinforcing properties of ethanol. These procedures include the previously-mentioned OSA, intravenous self-administration (IVSA), as well as intra-gastric and intra-cranial administration of ethanol. Because intra-gastric and intra-cranial routes of administration have been assessed in very limited amounts and models, these methodologies were not included here. IVSA has also received somewhat limited publication but is included here, despite the failure of this approach in rat models (Hyytia et al., 1996), in order to address the lack of data on this behavioral model in the literature as well as bring attention to the positive aspects of this model for addressing the reinforcing properties of ethanol.

The main purpose of this critical synthetic review, however, is to use the technique of genetic correlation to assess the relationship between free-choice consumption of ethanol and methods using operant and classical conditioning to assess ethanol's reinforcing actions. By combining the broadest possible literature on genetic variation we sought to detect consistent relationships among drinking and other behavioral models of alcohol's rewarding and aversive effects such as OSA, CPP, and CTA.

Materials and Methods

A literature search was conducted using PubMed (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed), and is current as of February 8, 2007. The search string was as follows, where * indicates the wild card:

> ((transgenic OR knockout OR inbred AND strain) OR (select* AND breeding OR bred)) AND ((operant OR self-administration) OR (condition* AND place) OR (condition* AND taste)) AND (ethanol OR alcohol)

Only papers that covered mouse or rat species and were written in English were used, yielding 182 results. From there, the author included papers that met the following criteria: First, each animal model (i.e., pair of selected lines, panel of inbred strains, or pairing of wild-type (WT) and genetically altered mouse) must have had at least one paper among the search results classifying the model on 24-hour free choice drinking with a two-bottle choice paradigm of water and drinking an unsweetened concentration of ethanol that fell between 8 and 12% v/v ethanol. The majority of papers listed here used 10%, however, to be more representative of the literature, a range was employed in order to also include those few papers using concentrations slightly outside 10%. This concentration range was chosen because of its popularity within the literature and its presumed ability to allow animals to encounter the pharmacological effects of ethanol in comparison with lower (e.g., 3%) concentrations. Furthermore, there must have been a finding of a genetic difference in free-choice drinking, or the paper would not be able to speak to our central question, which was whether such differences predict differences in other phenotypes.

Next, each animal model must also have had a minimum of one published result, same or separate from the drinking paper, which classified the model in terms of performance on ethanol CTA, ethanol place preference (in mice) or aversion (in rats), ethanol OSA, or IVSA of ethanol. For example, the High-(HARF) and Low-Addiction Research Foundation (LARF) ethanol-preferring rats selectively bred under limited-access conditions were not included in this paper because, while they have been assessed under 24hr 2-bottle choice drinking (Le et al., 2001), the author was unable to locate other papers assessing this genetic model in the behavioral tasks listed above. Likewise, while papers concerning High Alcohol Withdrawal (HAW) and Low Alcohol Withdrawal (LAW) models regarding CPP and CTA were located (Chester et al., 1998), the author was unable to locate published data regarding 24-hour two-bottle choice drinking.

Finally, models such as knockouts and transgenics must have included a description of the background genetic stock and this genetic background must match between papers. If this was not the case, or was unclear from the published articles, the paper would be eliminated from this review. For example, a paper assessing a μ opioid knockout mouse model on a C57BL/6J background would not be compared to a paper assessing a similar μ opioid knockout mouse model but on a C57BL/6×129SvEv background. This limit was imposed based on a range of literature suggesting that the background genes of a transgenic or knockout model can greatly influence the outcome of such behavioral measures (Bailey et al., 2006; Linder, 2006; Wolfer et al., 2002; Yoshiki and Moriwaki, 2006).

Replicated selected lines are each listed separately, as each pair is a separate assessment of whether a genetic correlation between the phenotype in question and alcohol drinking is present. If a paper assessed a behavioral task in one model but did not assess this behavior concurrently with its comparison strain/phenotype/genotype, then the paper was excluded. This was the case in Blizard and McClearn (2000), for example, where drinking was assessed in DBA/2J mice but not C57BL/6J mice, and CTA was assessed in C57BL/6J mice but not DBA/2J mice.

The remaining papers were then organized by model in a table (see Table 1) and thoroughly assessed for the traits represented in the paper. Genetic models were first ordered by listing the higher drinking counterpart first, followed by the lower drinking model. For example, if a High Alcohol Preferring (HAP) mouse drank more ethanol (20 g/kg) during a 24-hour two bottle choice paradigm than the Low Alcohol Preferring (LAP) mouse (1 g/kg), it would be listed as follows: HAP v LAP.

These models were then assessed on the other behavioral tasks pertinent to this review. We then utilized two meta-analysis techniques to combine the results. First, we used a non-quantitative assessment that looked for the direction of differences between genotypes. Thus, if HAP mice exhibited a significantly higher OSA response rate when reinforced with ethanol than LAP mice, the score given in that section of the table would appear as a "++". If the difference was not found to be significant statistically but there was an observable trend in a graph or a table, the score given was "+". Conversely, if a higher-drinking GIRK2 knockout mouse developed significantly less of a CTA to ethanol, compared to its lower-drinking, wild-type counterpart (WT), which developed a very pronounced CTA, they would be listed as GIRK2 v WT and the score would be "--". If there was a slight trend but this difference was not significant, a "-" was placed in that cell. When there were absolutely no observable differences between the genotypes, "ND" was placed in the cell. Empty cells denote lack of appropriate literature where no values were able to be reported. Second, when possible, we extracted a quantitative measure of the differences between genotypes, by deriving a t-value for each genetic comparison. Because data sets were not always presented with sufficient detail to extract such a measure, this more quantitative analysis could not be applied to all data sets.

Statistics

To examine the pattern of results of the qualitative analysis, we used the Fisher Sign Test meta-analysis. This results in a series of pairwise relationships between the differences seen in drinking and the differences (if any) see in the other phenotypes assessed. For analysis using the Fisher Sign Test, cells listed as ND were not included, and pairwise comparisons were done between drinking on the one hand, and each of the other phenotypes (CTA, CPP, and OSA) on the other, based on the remaining sample of genotypes. Therefore, the N for CTA was 15, for CPP 10, and for OSA 10. Statistical significance was evaluated based upon the critical values chart for the sign test (Marascuilo, 1971). The Fisher Sign Test analysis appears in Table 2.

For the quantitative analysis, we simply added the obtained t value for each of the genetic comparisons. Negative values of t were assigned when the obtained difference was in the opposite direction as that of drinking; the absolute value of the sum of positive and negative t values was then derived. We were able to do this with 10 CTA findings, 8 CPP findings, and 10 OSA findings (one ethanol place conditioning paper was not included in the final tally of t-values because unlike all others, the animals showed an aversion rather than a preference). To obtain a p-value for each comparison, we made a conservative assumption that degrees of freedom for the meta-analysis was 100; additionally, above this point, the critical value of t doesn't change substantially up to a degrees of freedom of infinity.

Additionally, although not appearing in the table, two panels of inbred strains (Belknap et al., 1993; Broadbent et al., 2002) were used to assess correlations between free-choice drinking and CTA via Pearson's R analysis. Three recombinant inbred strain panels (Cunningham, 1995; Phillips et al., 1994; Risinger and Cunningham, 1998) were also used to determine correlations between free-choice drinking and CTA as well as free-choice drinking and CPP. In keeping with previously suggested methods for analyzing genetic correlations using inbred strains (Crabbe et al., 1990), a Pearson's R was used to determine genetic correlations among strain means. Significance was considered at or below the 95% critical value. Scatter plots representing both recombinant inbred and inbred panels and their corresponding CTA data from a 2 g/kg dose of ethanol are also given (Figure 1A and B, respectively). Lastly, IVSA was not calculated due to the limited number of entries.

Results

Broadly stated, we observed consistent genetic evidence for a positive relationship between home cage drinking and OSA ($p < 0.0001$), and a negative relationship between home cage drinking and CTA ($p < 0.0001$). These associations are remarkably consistent in the literature, with only 2 CTA papers and 1 OSA paper in the opposite direction of the overall trend. There is little evidence for a consistent association between home cage drinking and CPP using the qualitative analysis, though there was evidence for such an association using the quantitative analysis ($p < 0.0001$). The somewhat contradictory pattern of findings between the quantitative and qualitative analysis for CPP arises from the fact that many of the papers showing somewhat lower CPP in high-drinking populations did not reach significance and therefore did not generate a t value for our quantitative analysis. One interpretation of this outcome is that these are spurious findings, and that there is a genuine association between CPP and drinking; another is that the larger segment of the literature covered by the qualitative analysis, showing no consistent trend, is the more reliable outcome. This latter interpretation is supported by the lack of an association between CPP and drinking in panels of inbred strains (see below). However, given that the qualitative analysis contains a sampling of non-significant findings, more confidence should ultimately be given to the quantitative analysis showing that increased drinking is associated with increased CPP behavior. There were too few data points to determine associations with IVSA. A qualitative index of all animal models that fit the criterion for this paper, except the inbred panel and recombinant inbred panels, appears in Table 1.

Recombinant inbred strain panels showed no significant correlations between free-choice drinking and two other measures of ethanol's hedonic effects, CTA and CPP. Specifically, ethanol drinking (Phillips et al., 1994; Rodriguez et al., 1994) was not correlated with 2 g/kg ethanol CPP (Cunningham, 1995) for either of the two drinking papers: the largest absolute r-value of the correlation was 0.261. CTA and drinking were not correlated, either. In the CTA study with recombinant inbred strains, two doses of ethanol were used, 2 g/kg (see Figure 1A) and 4 g/kg (Risinger and Cunningham, 1998). Neither dose resulted in a significant correlation with free choice drinking of 10% ethanol ($r = 0.119$ for 2 g/kg and $r = 0.324$ for 4 g/kg).

Conversely, a panel of inbred strains did show a correlation between CTA following a 2 g/kg ethanol dose (see Figure 1B) and free choice drinking (Cunningham, 1995; Phillips et al., 1994). This genetic correlation ($r = 0.623$, n=13; $p < 0.05$) agreed strongly with previously published correlations between CTA at 2 g/kg and ethanol preference (Belknap et al., 1993; Broadbent et al., 2002). This association between CTA and drinking supports the finding of such an association in both the quantitative and qualitative meta-analyses described above. However, it should be noted that the correlation between a 4 g/kg CTA dose of ethanol and ethanol consumption missed significance ($r = 0.508$).

Discussion

After genetic analysis, there is substantial agreement here that populations which self-administer ethanol in a free-choice access paradigm also work for ethanol in operant studies. This parallel indicates that free-choice alcohol consumption in the home cage may indicate greater ethanol reinforcement when measured by operant oral self-administration, both in selected lines and in populations for which other methods for creating genetic change were used, including targeted mutations. We observed this association despite important procedural differences between home cage drinking and operant self-administration, including the requirement for a response (other than drinking) to obtain ethanol in an operant setting, the fact that assessment of operant behavior is typically conducted during a defined time period each day in an operant chamber (instead of assessing behavior for 24 hours a day in the home cage), and the fact that operant studies often break up free-choice drinking behavior into small

sips that typically serve as a reinforcer. Agreement in findings between these divergent behaviors suggests that they share genetic and neurobiological mechanisms.

One way to think about the similar genetic mechanisms underlying home cage drinking and operant oral self-administration is that they are both, in fact, consummatory behaviors. As suggested by Samson and colleagues based upon a fine-grained temporal analysis of operant responding for ethanol (Samson et al., 2000), there is a great degree of similarity between intake of ethanol from a sipper tube (i.e., a completely consummatory response) and intake of animals responding for small quantities of ethanol, each interspersed by a relatively small string of operant responses. A procedure in which animals emit all of their responses at the beginning of a session, followed by uninterrupted access to ethanol, may yield better understanding of behavior which is not interchangeable with consummatory responses (Czachowski and Samson, 1999; Grahame and Grose, 2004).

We were also able to observe a consistent relationship between ethanol CTA and home cage ethanol consumption, both in the meta-analyses depicted in Table 1 and in the inbred strain panels at the 2 g/kg dose (see Figure 1B). We found that higher home cage alcohol drinking associates with lower CTA, agreeing with previous findings that strains of mice which show stronger taste aversion also tend to show lower ethanol preference and higher withdrawal severity (Broadbent et al., 2002). Such results suggest that with respect to ethanol, CTA reflects the likelihood of animals to acquire an aversion to flavors (including ethanol itself) paired with ethanol's actions, an effect that interferes with drinking behavior.

In spite of the fact that CTA produces taste avoidance, there are several theories that interpret this behavior as indicative of a reinforcing effect of the drug US. Most self-administered drugs produce CTA (Grigson, 1997; Hunt and Amit, 1987), and this finding is further supported with this review. CTA has been hypothesized previously to be positively correlated with sensitivity to drug reinforcement (Hunt and Amit, 1987). According to this hypothesis, taste aversion is caused by the same appetitive drug effects that mediate self-administration. It is this positive rewarding drug state, rather than some aversive effect of drugs that are self-administered, that will lead to suppression of intake due to a conditioned "taste shyness." In a similar direction, the resulting taste avoidance may be due to a comparison between the less valued flavor stimulus and the anticipation of a highly valued drug effect when saccharin serves as the CS, as was often (but not always) the case in the reviewed experiments (Grigson and Freet, 2000). Such an interpretation is hard to maintain, however, when one considers that populations such as DBA/2J mice, and selectively bred NP rats and LAP mice, which drink almost no alcohol in the home cage, show robust conditioned taste aversion to ethanol. One would have to speculate that these populations are so highly reinforced by ethanol that they drink almost none of it, an interpretation that seems implausible. Additionally, the Broadbent et al. (2002) study explicitly avoided a saccharin CS (opting for a salt flavor) to discourage any successive contrast interpretation of their findings. All in all, the more parsimonious explanation of the present findings is that CTA with an ethanol US is, in fact, mediated by aversive properties of that US, which also tend to discourage free-choice alcohol consumption.

Notably, both the inbred strain panel in Figure 1B as well as the genetic analysis we offered in Table 1 support an association between CTA and ethanol intake, while the recombinant strain panel examined in Figure 1A does not. While it is possible that this difference could reflect a true weakness of this association, a perhaps more likely reason that association was not observed in the recombinant inbred strain panel is that these panels are made of populations that are descended from just two inbred parent strains. As such, they can only have two alleles at any locus at which the parent strains differ. This may limit the ability to generalize results from these populations (Crabbe et al., 1990). Inbred strain panels, as well as the genetic analysis technique we used in Table 1, do not suffer from this shortcoming because they use genetically

very diverse populations. Therefore, these techniques may have more power to detect genetic associations that truly exist, providing that there are enough differing populations in the analysis.

We observed a link between home cage drinking and CPP, albeit one with somewhat more heterogeneity throughout the literature for this phenotype than for the others, both currently and historically. There was support from the quantitative analysis for this connection, but in the qualitative analysis and the recombinant inbred strain panel, we observed a near-normal distribution of findings. In CPP, a change in the amount of time spent in the environment paired with the drug is thought to indicate acquisition of an association between that environment and the effects of the drug. If a preference for the environment is observed, the drug is presumed to be rewarding, while in contrast, if avoidance of the environment associated with the drug is seen, the drug is thought to produce dysphoric effects. Overall results suggest that CPP is greater when animals voluntarily consume more alcohol, in line with the idea that both of these behaviors are related to the reinforcing effects of ethanol.

However, this result is complicated by the fact that although mice typically show a preference for ethanol-paired environments, rats typically show an aversion. When CPP was initially used to test for reinforcing or aversive effects of ethanol in rats, Black et al. (1973) concluded that the central effects of ethanol were reinforcing. This report proved over time to be controversial. Cunningham (1981) using the same parameters and dose later reported conditioned place aversion, suggesting that rats find ethanol aversive in this situation. Conditioned place aversion to ethanol for rats has subsequently been the most common finding, including the one rat place conditioning paper fitting the criterion for inclusion in the meta-analysis (Stewart et al., 1996). While this review integrates findings across both rats and mice, there may be some intrinsic biological differences in response to the reinforcing and aversive effects of ethanol existing between rats and mice. Ethanol-induced CPP is fairly well established among mouse literature (though notably lacking in the C57BL/6J mouse, the prototypical high-drinking strain; see Table 1), but the condition under which rats will exhibit an ethanol CPP remains unclear (Cunningham and Henderson, 2000; Fidler et al., 2004; Tzschentke, 1998). Fidler et al. (2004) discusses the role route of administration plays on the development of CPP. Through the implementation of an intragastric route of administration, they support the conclusion that it does not play a role, in contrast with previous work that suggested that the route of administration did play a role in selected lines of rats (Ciccocioppo et al., 1999). Furthermore, it has also been proposed (Cunningham et al., 2002; Cunningham and Henderson, 2000) that both CPP, conditioned place aversion, and CTA can be produced in the same animal by the same dose of ethanol simply by varying the timing of administration of the ethanol and the nature of the cues paired with ethanol delivery (gustatory or tactile/environmental). One recent study indicated a genetic correlation between CTA and place aversion conditioning wrought by injecting ethanol after exposure to the CS, suggesting that these two phenotypes can be related when measuring the aversive effects of ethanol (Cunningham and Ignatoff, 2000).

Although there are relatively few papers assessing intravenous self-administration, one of the targets of this review, these data seem to suggest that it, like CPP, may not be closely related to home cage drinking. For example, C57BL/6J mice, which self-administer more in free-choice, as well as operant paradigms, than a common comparison inbred strain, the DBA/2J mice, seem to not differ from that strain in intravenous self-administration (Grahame and Cunningham, 1997). However, beta endorphin knockout mice, which do not drink more ethanol than their wild-type counterparts, self-administer ethanol to a greater extent intravenously than wild-types (Grahame et al., 1998). While these are interesting findings, more work is called for in this area of research. Perhaps the lack of IVSA literature may not be surprising, considering that this method is much more time consuming and technically-challenging compared to others mentioned here, and given the fact that ethanol is typically orally self-

administered in humans. However, IVSA has long been used in the study of other drugs of abuse, and has numerous advantages compared to other methods of assessing the reinforcing properties of drugs. The most important of these with respect to ethanol is the ability of this procedure to easily bypass preingestive factors such as taste and smell, which may interfere with ethanol self-administration in populations that otherwise find the pharmacological effects of ethanol rewarding (for reviews of this literature, see Thomsen and Caine, 2007 and Yokel, 1987).

When new genetically modified populations come into play on an almost daily basis, many of which are potentially related to ethanol's rewarding effects, researchers tend to choose behavioral assays that are both easy to perform and are interpretable. The current review suggests that home cage drinking is genetically correlated with other behavioral assays - OSA, CTA, and CPP - that rely on operant and classical conditioning. Although these other measures are important, one might argue based on the present data that they do not contribute greatly, genetically speaking, to what one already knows after assessing home cage drinking alone. However, several sets of data that converge on the same conclusion increase confidence in genetic differences, and a genetic difference seen both in drinking and a more formal behavioral test such as OSA, CTA, or CPP should be treated as more robust than a difference seen in drinking alone. Notably, after detecting a difference in home cage drinking, it may be somewhat easier to detect similar differences in OSA and CTA as opposed to CPP, which showed a more modest association with home cage drinking.

Finally, these animal studies may be further extended to compare with the human literature on human family history-positive studies on alcoholism. Research on genetic variations such as dopamine D2 and D3 receptors, as well as the cannabinoid and opioid receptor systems (Blum et al., 1995; Bowirrat and Oscar-Berman, 2005; Manzanares et al., 2005; Oswald and Wand, 2004) have begun to uncover genetic influences in alcoholism in humans. Through the use of animal research and animal models such as selective breeding, transgenic and knockout models, as well as inbred strains, we may begin to gain insight into the human condition and develop treatments or prevention strategies to help control, treat, and manage alcoholism.

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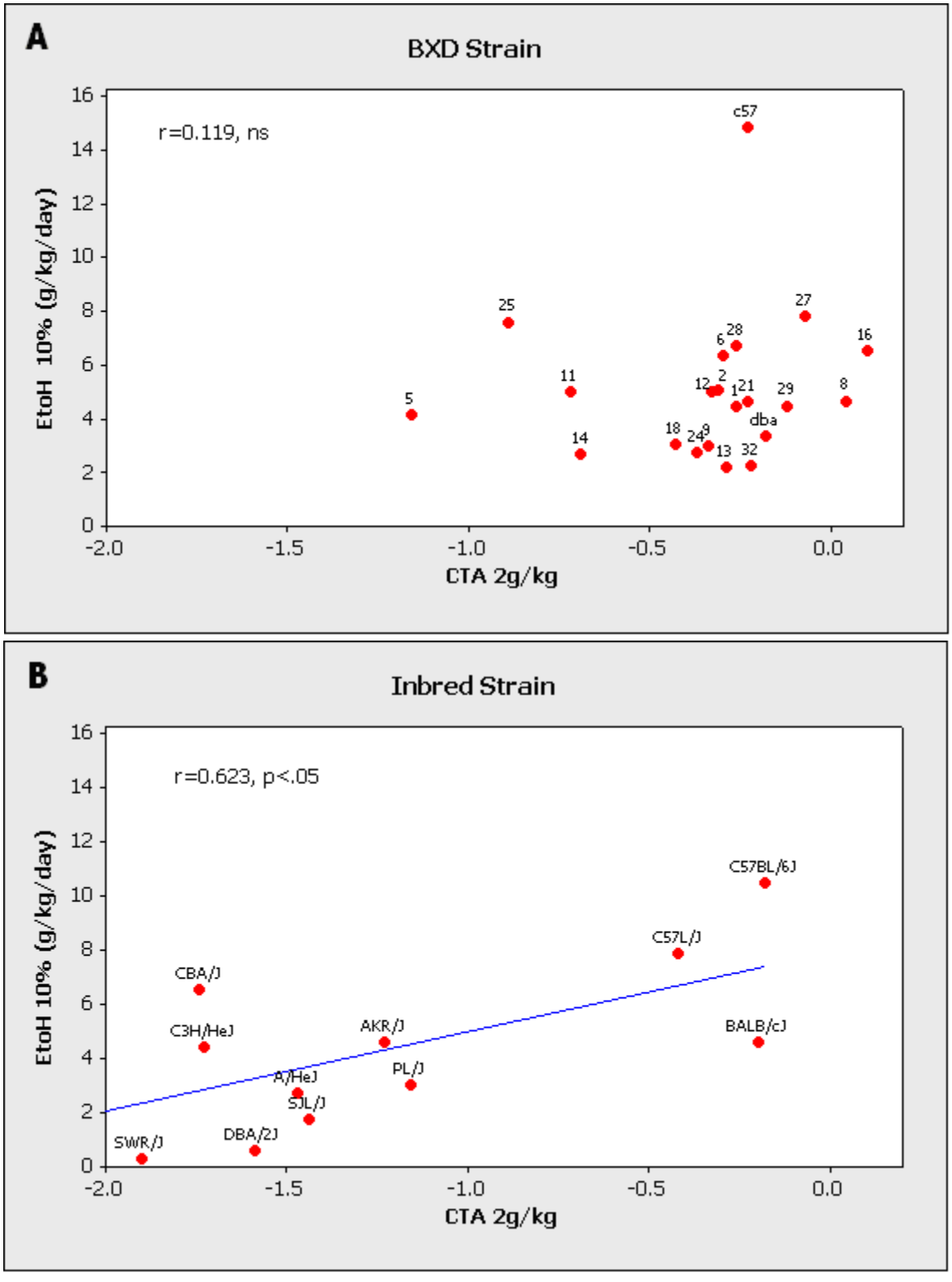

Figure 1.

Scatter plots representing g/kg scores taken from Rodriguez et al., (1994) and Belknap (1993), and CTA scores taken from Risinger and Cunningham (1998) and Broadbent et al.,

(2002), respectively. Inbred strains (Panel B) showed a significant positive correlation with development of CTA and home cage drinking (as measured by decrease in g/kg intake), while the recombinant inbred strains (Panel A) did not.

Table 1

A list of genotypes used in the meta-analysis. Each model is listed with the higher drinking animal listed first. Subsequent columns indicate performance differences on conditioned taste aversion (CTA), conditioned place preference (CPP), operant oral self-administration (OSA) or operant conditioning intravenous (OCIV). Each column indicates a subjective rating of differences between genotypes. A score "+ +" signifies a significant difference in the same direction as the drinking difference between the two comparison lines. If the difference was not significant statistically but there was an observable trend in a graph or a table, the score given was "+". The "--" and "-" signs indicate similar differences, but in the opposite direction. No difference is denoted with (ND). The Derived T column indicates the value of a t-statistic for the difference between the lines in comparisons where significant differences were present in the literature and a t-value was able to be derived. A positive t score indicates a difference between genotypes in the same direction as the drinking difference, while a negative t score indicates a difference between the genotypes in the opposite direction as the drinking difference. Other abbreviations are as follows: HAP/LAP = High- and Low-Alcohol Preferring; STDRHI/STDRLO = short term selection for high and low drinking; SS/LS = Short and Long Sleep; WSR/WSP = Withdrawal Seizure Prone and Resistant; LTA/HTA = Low and High Taste Aversion; P/NP = Preferring and Non-Preferring; HAD/LAD = High and Low Alcohol Drinking; AA/ANA = Alcohol Alcohol and Non-Alcohol; sP/sNP = Sardianian Preferring/Non-Preferring; TAR/TAP = Taste Aversion Prone and Resistant; gen = generation; progen= progenitor stock; WT = wild type; KO= knock out; OE= over-expressing; HT= heterozygous

	Animal Used	Background	CTA	CTA dose(s)	Derived T	CPP	CPP dose(s)	Derived T	OSA	OSA conc(s)	Derived T	OCIV	OCIV doses	Citation
inbred mice	C57BL/6J v DBA/2J	Inbred	--	3.0, 4.0 g/kg	-16.84	--	2.0 g/kg	-1.97	++	10%	4.06	+	60, 75, 90 mg/kg	(Bachtell et al., 2003; Belknap et al., 1977; Broadbent et al., 1996; Cunningham, 1995a; Grahame and Cunningham, 1997; Meliska et al., 1995; Risinger et al., 1998; Risinger and Cunningham, 1995)
	C57BL/6J v BALB/cJ	Inbred							++	1.25-40%	8.15			(Elmer et al., 1987)
single gene alterations in mice	WT v CB1 KO	CD1 F15				++	1.0, 1.5, 2 g/kg	3.09						(Houchi et al., 2005; Naassila et al., 2004)
	nTG v CRF TG-OE	bc C57BL/6J 9 gen	+	1.5, 3.0 g/kg										(Palmer et al., 2004)
	CRF KO v WT	bc C57BL/6J 8 gen	+	2.0, 4.0 g/kg										(Sharpe et al., 2005)
	WT v D2 KO	C57BL/6J				++	2.0 g/kg	3.29	++	10%	3.08			(Cunningham et al., 2000; Phillips et al., 1998; Risinger et al., 2000)
	WT v α1 GABAA KO	C57BL/6×129SvEv background	-	2.5 g/kg		-	2.0 g/kg							(Blednov et al., 2003)
	WT v μ opioid KO	mixed C57/129sv background				++	2.0 g/kg	2.05	++	10%	2.12			(Hall et al., 2001; Roberts et al., 2000)
	GIRK2 KO v WT	C57BL/6J × 129/SvJ background	--	2.0, 2.5, 3.0 g.kg	-2.95	--	2.0, 3.0 g/kg	-2.29						(Blednov et al., 2001; Hill et al., 2003)

	Animal Used	Background	CTA	CTA dose(s)	Derived T	CPP	CPP dose(s)	Derived T	OSA	OSA conc(s)	Derived T	OCIV	OCIV doses	Citation
	Het v Dbh KO	129/SvEv × C57BL/6J background	-	2.0 g/kg										(Weinshenker et al., 2000)
	Fast v Slow REP 1	HS/Ibg progen; gen 14-17	--	2.5 g/kg	-2.05	ND	2.0 g/kg		ND	8%				(Risinger et al., 1994; Sanchez et al., 1996)
	Fast v Slow REP 2	HS/Ibg progen; gen 14-17	--	2.5 g/kg	-4.72	ND	2.0 g/kg		ND	8%				(Risinger et al., 1994; Sanchez et al., 1996)
	HAP v LAP REP 1	HS/Ibg progen; gen 7, 13, 16	--	2.0, 4.0 g/kg	-2.3	-	1.5, 3.0, 4.0 g/kg							(Chester et al., 2003; Grahame et al., 2001)
	HAP v LAP REP 2	HS/Ibg progen; gen 7, 13, 16	--	2.0, 4.0 g/kg	-2.3	-	1.5, 3.0, 4.0 g/kg							(Chester et al., 2003; Grahame et al., 2001)
	STDRHI v STDRLO	B6D2F2, gen 4	-	4 g/kg		++	2 g/kg	2.39						(Phillips et al., 2005)
	SS v LS	Heterogenous stock selective breeding to gen?							--	8%	-3.41			(Collins, 1981; Elmer et al., 1990; Fuller 1980)
	COLD v HOT	HS/Ibg progen; gen 12-14	--	2.25 g/kg	-5.40	--	2.25 g/kg	-2.75						(Cunningham et al., 1991)
	WSR v WSP	gen 53	ND	2.5, 4.0 g/kg		ND	2.0 g/kg		ND	8%				(Barbera et al., 1994; Chester et al., 1998; Crabbe et al., 1992; Kosobud et al., 1988)
selectively bred mice	LTA v HTA	B6D2F2 selective breeding to S-2	-	4 g/kg		ND	2 g/kg							(Phillips et al., 2005)
	P v NP	8, 25, 31-33 gen	--	0.25, 0.5, 1.0,1.87 g/kg		CPA	-- 0.5, 1.0, 1.5 g/kg	3.01	++	8%, 10%	2.61			(Froehlich et al., 1988; Murphy et al., 1989; Penn et al., 1978; Ritz et al., 1994; Samson et al., 1998; Schechter, 1992; Stewart et al., 1996)
	HAD v LAD	26 gen I							++	10%	5.25			(Ritz et al., 1994; Samson et al., 1998)
	HAD v LAD	22 gen II							++	10%	5.25			(Ritz et al., 1994; Samson et al., 1998)
selectively bred rats	ALKO AA v ANA	?	ND	1.5 g/kg		ND	2.0 g/kg		++	8%	3.93			(Gauvin et al., 2000; Ritz et al., 1986)

	Animal Used	Background	CTA	CTA dose(s)	Derived T	CPP	CPP dose(s)	Derived T	OSA	OSA conc(s)	Derived T	OCIV	OCIV doses	Citation
	sP and sNP	gen 50	--	0.5, 1.0 g/kg	-3.59				++	10%	4.27			(Brunetti et al., 2002; Vacca et al., 2002)
	TAR v TAP	23, 35-36 gen	--	0.25, 0.75, 1.25 g/kg	-3.8									(Elkins et al., 1992; Orr et al., 2004; Orr et al., 1997)
	M520 v WKY	?	--	0.5, 1.0, 1.5 g/kg	-2.07									(Cannon and Carrell, 1987)
				Sum of t-values	-46.02			5.78			35.31			

Table 2

Meta-Analysis of Ethanol Reward-Related Behaviors

Summary of the results in Table 1. The "Same" and "Opposite" cells were subjected to a Fisher Sign Test analysis, the results of which are on the last row of the table

	CTA	CPP	OSA	IVSA	TOTAL
Same (++ or +)	2	4	9	1	
Opposite (-- or -)	13	6	1	0	
No Difference (ND)	2	5	3	0	
TOTAL	**17**	**15**	**13**	**1**	**46**
Fisher's Sign Test	$p \leq 0.03$	$p > 0.75$,ns	$p \leq 0.03$	N/A	