Published in final edited form as:
Alcohol. 2014 May ; 48(3): 287–293. doi:10.1016/j.alcohol.2013.11.006.

Alcohol Dependence and Free-Choice Drinking in Mice

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Abstract

Alcohol dependence continues to be an important health concern and animal models are critical to furthering our understanding of this complex disease. A hallmark feature of alcoholism is a significant increase in alcohol drinking over time. While several different animal models of excessive alcohol (ethanol) drinking exist for mice and rats, a growing number of laboratories are using a model that combines chronic ethanol exposure procedures with voluntary ethanol drinking with mice as experimental subjects. Primarily, these studies use a chronic intermittent ethanol (CIE) exposure pattern to render mice dependent and a 2-h limited access procedure to evaluate drinking behavior. Compared to non-dependent mice that also drink ethanol, the ethanol-dependent mice demonstrate significant increases in voluntary ethanol drinking. The increased drinking significantly elevates blood and brain ethanol concentrations compared to the non-dependent control mice. Studies report that the increased drinking by dependent mice is driven by neuroadaptations in glutamatergic and corticotropin-releasing factor signaling in different brain regions known to be involved in alcohol-related behaviors. The dysregulation of these systems parallels findings in human alcoholics and treatments that demonstrate efficacy in alcoholics can also reduce drinking in this model. Moreover, preclinical findings have informed the development of human clinical trials, further highlighting the translational potential of the model. As a result of these features, the CIE exposure and free-choice drinking model is becoming more widely used and promises to provide more insight into mechanisms of excessive drinking that may be important for developing treatments for human alcoholics. The salient features and possible future considerations for CIE exposure and free-choice drinking in mice are discussed.

Keywords

ethanol; dependence; chronic intermittent exposure; limited access; mouse; glutamate; CRF

Introduction

Heavy alcohol (ethanol) consumption remains a serious public health problem in the United States and worldwide (Grant et al., 2004; Mokdad, Marks, Stroup, & Gerberding, 2004)

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(http://www.who.int/gho/alcohol/en/). Long periods of heavy ethanol consumption lead to ethanol dependence that is accompanied by neuroadaptive changes in the brain that may perpetuate continued drinking, despite serious personal consequences. Unfortunately, effective treatments for alcoholism as well as a comprehensive understanding of the neurobiological underpinnings of this complex health problem are elusive. Therefore, animal models that incorporate free-choice ethanol drinking as a behavioral outcome are crucial for examining not only the effects of different therapeutics on ethanol drinking, but also understanding how the brain adapts to chronic ethanol exposure and how these adaptations may promote more consumption. While there are a variety of procedures capable of engendering high levels of ethanol drinking in experimental rodents (Becker, 2013), a growing number of studies have used ethanol-dependence procedures in conjunction with free-choice drinking (Becker & Lopez, 2004; Dhaher, Finn, Snelling, & Hitzemann, 2008; Finn et al., 2007; Griffin, Lopez, & Becker, 2009; Hansson, Rimondini, Neznanova, Sommer, & Heilig, 2008; Jeanes, Buske, & Morrisett, 2011; Lopez & Becker, 2005; Sommer et al., 2008), or operant self-administration procedures (Chu, Koob, Cole, Zorrilla, & Roberts, 2007; Fidler, Clews, & Cunningham, 2006; Fidler et al., 2012; Gilpin, Richardson, & Koob, 2008; O'Dell, Roberts, Smith, & Koob, 2004; Richardson, Lee, O'Dell, Koob, & Rivier, 2008; Roberts, Cole, & Koob, 1996) in both mice and rats to investigate these important issues. Importantly, these procedures reliably increase ethanol intake in both species and these procedures are being widely used to investigate different aspects of dependence-induced increases in ethanol drinking.

As a research tool, mice play an important role because of the wide range of existing transgenic and inbred strains and the relative ease of generating very specific mutations necessary for some mechanistic investigations. Moreover, although mice will readily press levers to obtain access to ethanol (Chu et al., 2007; Griffin, Nguyen, Deleon, & Middaugh, 2012), the natural avidity that some mouse strains have toward ethanol produces enough consumption that free-choice, limited access drinking procedures in the home cage are possible. This is an important characteristic, because unlike continuous access procedures, limited access procedures allow correlations to be established between the amount of ethanol intake and blood ethanol concentrations. Further, free-choice drinking procedures with mice in the home cage can be easily implemented because there is no special equipment needed and, consequently, can be conducted on a large scale, which may be important for some experiments. While free-choice drinking procedures do not allow assessment of the reinforcing efficacy of ethanol that is possible with operant self-administration procedures, a recent meta-analysis of the literature found a strong, positive concordance between amounts of home cage drinking and operant self-administration (Green & Grahame, 2008), suggesting that ethanol drinking in the home cage is driven by ethanol reinforcement. Considering these positive features, this review focuses on the use of free-choice drinking in mice as the behavioral outcome in models of ethanol dependence.

Free-choice drinking in mice

In free-choice drinking models, mice are usually presented with 2 bottles during the ethanol access period, one containing diluted ethanol (e.g., 15% v/v) and the other containing water, providing a choice to the mice. The amount of ethanol solution or water consumed is easily

determined by comparing the amount of fluid in the bottles before and after the access period. With care, fluid lost during handling by the experimenter or evaporation can be minimal but can be easily estimated by including sets of bottles on empty cages. Therefore, an advantage of free-choice drinking procedures is that fluid volumes consumed by the mice can be accurately measured. In turn, a preference score can be determined by comparing the volumes of the ethanol solution consumed versus the water consumed. Calculating ethanol preference can be valuable when different mouse strains are compared because preference can vary widely across strains (Belknap, Crabbe, & Young, 1993; Rodgers, 1972; Rodgers & McClearn, 1964).

An important variable in free-choice drinking is the duration of the access period. Depending on the goal of the study, the access period used by different investigators can vary widely in free-choice situations, ranging from as little as 30 min to continuous 24-h access. With regard to ethanol dependence models, most of the available studies published so far have used limited access periods of 2 h, although a recent study showed that vapor inhalation procedures could increase drinking in mice given 24 h of access (Depoy et al., 2013). An important advantage of the 2-h duration of access is that it is generally long enough to allow a reasonable level of ethanol intake so that both increases and decreases can be accurately measured. Additionally, a 2-h period is short enough to establish significant correlations between the amount of ethanol consumed (g/kg) and blood ethanol concentrations (BECs) (Becker & Lopez, 2004; Dhaher et al., 2008; Finn et al., 2007). With longer access periods, it can become more difficult to establish a significant relationship between the amount of intake and the BEC because there is greater variability in elapsed time between the last drinking bout during the access period and the blood collection. Measuring post-access BECs is important because it confirms that ethanol was actually consumed and that the mice encountered the pharmacological effects of ethanol, rather than the ethanol simply being spilled because mice played with the drinking spout on the bottle. With limited access, a decision must also be made regarding when the drinking period will occur. For example, some studies place the bottles on the home cage 30 min prior to lights-out (Becker & Lopez, 2004), while other studies have waited until 3 h into the dark phase to allow access (Finn et al., 2007). In either case, investigators are taking advantage of the inclination of mice, which are nocturnal, to initiate a major feeding and drinking episode near the beginning of the dark phase to maximize ethanol intake.

An additional variable to consider is the concentration of ethanol presented to the mice. While a variety of different concentrations of ethanol are consumed by mice, most of the available studies using dependence procedures and free-choice drinking have employed 15% (v/v) ethanol. A recent study did use 10% ethanol and showed significant increases in voluntary consumption by ethanol-dependent C57BL/6J (B6) mice compared to non-dependent mice (Lopez, Grahame, & Becker, 2011). For B6 mice, ethanol concentrations ranging from 10-15% produce amounts of intake in a 2-h limited access session in the range of 1-3 grams per kilogram (g/kg), which allows significant increases in drinking to be achieved. At the same time, ethanol intake by non-dependent B6 mice within this range of concentrations is generally large enough that it is sensitive to decreases without concern for a “floor” effect, an important consideration for evaluating pharmacotherapies expected to reduce free-choice drinking in a dose-dependent manner (Becker et al., 2013; Griffin et al.,

2012). Thus, choosing a concentration of ethanol for the free-choice access period requires consideration of the expected experimental outcomes.

Another issue that arises with free-choice drinking is determining when mice are consuming ethanol during the access period. Temporal patterns of ethanol consumption can be tracked using lickometers (Ford, Nickel, & Finn, 2005; Ford, Nickel, Phillips, & Finn, 2005; Griffin, Lopez, Yanke, Middaugh, & Becker, 2009; Griffin, Middaugh, & Becker, 2007; Sharpe & Samson, 2003). With this type of equipment, the drinking bottles and the floor of the cage are part of an electrical circuit that closes every time the mouse is positioned on the floor under a bottle and licks the solution in that bottle. The licks are counted by a computer and at the end of the access period, the total licks at the bottle should positively correlate with total ethanol intake. The computer also tracks the temporal pattern of licking, a feature that can be used to determine whether an experimental manipulation shifts ethanol consumption within the session, for example by indicating more intake earlier rather than later in the session. The temporal pattern of licking can also predict when brain ethanol concentrations may reach a peak because high licking rates precede increases in brain dialysate ethanol levels (Griffin, Lopez, Yanke, et al., 2009; Griffin et al., 2007). Finally, lickometers can be used to confirm preference for ethanol. For example, ethanol-preferring B6 mice consume very little water during 2-h limited access periods and lickometers confirmed this because few licks were registered at the water bottle (Griffin, Lopez, Yanke, et al., 2009). However, while lickometers do provide very important information about patterns of ethanol intake, their use may not be practical in every experiment because of the cost involved and the daily amount of time required to attach/unattach bottles to the system. An additional consideration is that there is anecdotal evidence indicating that lickometers must be used from the very first ethanol access period since it has been observed, at least in B6 mice, that ethanol consumption can decrease if lickometers are introduced after baseline drinking has been established (M. F. Lopez, personal communication). Thus, the use of lickometers must be carefully considered to determine if the information provided by their use will enhance the primary outcome measure of ethanol consumed.

Establishing ethanol dependence using chronic intermittent ethanol exposure

In laboratory rodents, ethanol dependence is established using procedures that generate enough exposure to elicit physical withdrawal symptoms when ethanol exposure ceases. One well-established procedure to accomplish this goal is the Decarli-Lieber liquid diet (DeCarli & Lieber, 1967), a diet in which the sole source of nutrients and water provided to the rodents also contains a significant percentage of ethanol. With enough exposure, the liquid diet produces dependence and has been shown to increase operant self-administration in mice (Chu et al., 2007) and rats (Gilpin et al., 2009). Another strategy that can be used to render rodents dependent and cause increased ethanol intake is the use of intragastic catheters. Fidler and Cunningham have revived a technique developed by Deutsch in the 1970s using intragastric catheters for ethanol delivery, which bypasses orosensory cues that might impede high levels of ethanol intake. These investigators have shown that passive infusions of ethanol by an intragastric catheter are capable of rendering rodents ethanol-dependent and subsequently increasing self-infusions of ethanol compared to those not experiencing passive infusions of ethanol (Fidler et al., 2006; Fidler et al., 2011; Fidler,

Oberlin, Struthers, & Cunningham, 2009; Fidler et al., 2012). However, in recent years, the procedure most often used to render mice and rats dependent on ethanol and to increase free-choice drinking or operant self-administration is the use of vapor inhalation procedures akin to those developed in the early 1970s (Goldstein & Pal, 1971).

A clear benefit of vapor inhalation procedures is the precise control over both the amount and duration of ethanol exposure. Essentially, by placing mice into an ethanol vapor chamber, the investigator is "clamping" blood ethanol concentrations at a specific level for a specified period of time. Thus, the intensity and duration of ethanol exposure can be tailored to the needs of the experiment. Currently, there are 2 patterns of vapor inhalation exposure of ethanol that have been shown to produce significant increases in voluntary ethanol intake in mice, which were adapted from prior work investigating ethanol withdrawal using handling-induced convulsions in mice (Becker & Hale, 1993). One pattern is a continuous exposure lasting 64 h (1 cycle) and the other is an intermittent pattern that also sums to 64 h, but which is broken up in four 16-h bouts of exposure separated by withdrawal periods of 8 h. While both patterns of exposure produce significant increases in ethanol drinking, the intermittent pattern of exposure produces the most robust and sustained increases in voluntary ethanol drinking, especially when repeated for multiple cycles (Lopez & Becker, 2005). This particular procedure is often called the chronic intermittent ethanol (CIE) exposure procedure and the robust increases in drinking indicate that the intermittent pattern of exposure is important. A recent report examined the possibility that other intermittent ethanol exposure procedures might also increase voluntary ethanol drinking in the 2-h, free-choice drinking procedure. The various possibilities were not exhaustively examined, but neither allowing 4 × 16-h access periods of free-choice drinking intermixed with limited access nor the use of oral gavage with high ethanol doses produced significant increases in voluntary ethanol consumption during the 2-h, free-choice drinking procedure (Griffin, Lopez, & Becker, 2009). As a result of the generally robust increases in ethanol drinking, the CIE exposure procedure has been used by several different laboratories using free-choice drinking as a primary behavioral outcome (Contet et al., 2011; Depoy et al., 2013; Dhaher et al., 2008; Finn et al., 2007; Griffin, Lopez, Yanke, et al., 2009b; Jeanes et al., 2011; Lopez & Becker, 2005).

Significant increases in voluntary drinking with CIE exposure procedures occur with as little as 1 cycle of exposure (Carrara-Nascimento, Lopez, Becker, Olive, & Camarini, 2013; Depoy et al., 2013; Dhaher et al., 2008; Griffin, Huan, Hazelbaker, Ramachandra, & Becker, 2013; Lopez et al., 2011), although the increase can be larger and more sustained with repeated cycles of exposure (Lopez & Becker, 2005). The ability of these procedures to demonstrate increases in free-choice drinking in a relatively short period of time was recently used to show that adolescent mice will also increase voluntary ethanol drinking when rendered dependent by CIE exposure (Carrara-Nascimento et al., 2013). Although the focus of this review is on free-choice drinking, it is important to note that CIE exposure itself is being used to investigate other aspects of ethanol dependence, e.g., fear conditioning (Holmes et al., 2012), dopamine function (Budygin et al., 2007; Healey, Winder, & Kash, 2008), gene expression changes (Contet et al., 2011; Melendez, McGinty, Kalivas, & Becker, 2012), NR2B subunit function (Wills et al., 2012), and tolerance (Lopez, Griffin, Melendez, & Becker, 2012). Indeed, these studies indicate that some investigators have

adopted the strategy of simply first determining whether ethanol dependence itself produces plasticity in a brain region or neurotransmission system of interest. Of course, these different aspects of dependence may also be important contributors to increased ethanol drinking by dependent mice and, if that is suspected, will require follow-up studies to determine whether the changes do influence free-choice drinking.

While undergoing ethanol vapor exposure, pyrazole must be used to inhibit ethanol metabolism in mice. This is in contrast to dependence studies in rats in which pyrazole does not need to be used to achieve blood ethanol concentrations of 150 to 200 mg% (Gilpin et al., 2009). Because mice can rapidly metabolize ethanol, pyrazole is critical for maintaining stable blood ethanol concentrations while mice are in the vapor inhalation chamber. It was recently demonstrated that pyrazole maintained stable BEC values throughout a 16-h exposure period whereas, when pyrazole was omitted, there were significant declines to near zero by the time mice were removed from the chamber (Griffin, Lopez, & Becker, 2009). Note that in ethanol-dependent mice, pyrazole is co-administered with a loading dose of ethanol prior to mice entering the vapor inhalation chamber. The non-dependent mice that serve as controls in these studies (i.e., receive air exposure) must also receive injections of pyrazole, but there is no loading dose of ethanol given to them.

Another consideration is that the vapor inhalation exposure procedures that work well in B6 mice may need adjustment to successfully produce increases in ethanol consumption with other strains of mice. For the B6 strain, it appears that adjusting the vapor inhalation chamber settings to achieve blood ethanol concentrations ranging from 175 to 225 mg/dL is optimal for producing increases in voluntary ethanol consumption (Griffin, Lopez, & Becker, 2009). Recent work suggests that this range may not be optimal for all strains of mice, though more work is needed. For example, ongoing work using a panel of BXD recombinant inbred stains of mice (the result of an F2 cross of C57BL/6 and DBA/2 strains) suggests that this range of ethanol exposure concentrations increases voluntary ethanol consumption in some BXD strains and reduces it in others (M. F. Lopez, personal communication). Similarly, a recent study using High-Alcohol Preferring (HAP) and Low-Alcohol Preferring (LAP) selectively bred lines of mice (based on high- and low-ethanol preference, respectively) found only the male mice of the replicate HAP-2 strain showed significant increases in voluntary intake when the ethanol exposure parameters where optimized for B6 mice (Lopez et al., 2011). For both the unpublished BXD findings and the HAP/LAP findings, further investigation is required to determine if vapor inhalation conditions can be found that elicit increases in drinking. Of course, if there are known differences in ethanol metabolism across strains it would be important that settings controlling vapor delivery to the chamber be adjusted differently to drive increases in voluntary consumption. Finally, it is important to consider the use of positive controls, e.g., C57BL/6J mice (Lopez et al., 2011), that would be expected to show increases in ethanol consumption under the conditions being used. A positive control can aid in interpreting the data if the experimental group does not produce a significant change from their own baseline drinking level.

Combining free-choice drinking and ethanol dependence

The most important feature of combining free-choice drinking with the CIE exposure procedures is that it produces significant increases in voluntary ethanol intake, modeling human alcoholic drinking. However, in order to elicit the increase in ethanol consumption by dependent mice, the mice must be introduced to ethanol for voluntary consumption before inducing dependence. If mice are first placed into the vapor inhalation chamber and then allowed access to ethanol for voluntary drinking, they will consume very little ethanol. The rejection of ethanol under these conditions may be because the positive, reinforcing effects of ethanol were not established prior to ethanol dependence (Becker & Lopez, 2004; Lopez et al., 2011). Interestingly, mice in the intragastric model self-infuse ethanol after a series of passive exposures without having prior ethanol experience (Fidler et al., 2011; Fidler et al., 2012), underscoring the idea that sensory cues (taste, smell) can play important roles in the initiation and maintenance of free-choice ethanol drinking by mice. On the other hand, rats have been shown to increase free-choice drinking in a post-dependent state in which their first experience with ethanol was actually in the vapor inhalation chamber (Hansson, Cippitelli, Sommer, Ciccocioppo, & Heilig, 2007; Molander et al., 2012; Sommer et al., 2008). These discrepancies in drinking in the post-dependent state between mice and rats notwithstanding, it is important to introduce mice to the rewarding properties of ethanol before rendering them dependent using a baseline period of free-choice drinking. While this “introductory” period of drinking could be considered a negative feature of the mouse model since it extends the duration of the experiment, it does provide a crucial foundation for the experiment by establishing a baseline level of ethanol drinking against which various experimental manipulations can be compared.

After stable baseline drinking is established and mice are rendered dependent, voluntary ethanol consumption will increase. In the typical 2-h limited access session, reports from different laboratories indicate that ethanol consumption can increase by 0.5 to more than 1 g/kg over baseline levels of intake. The increased consumption by dependent mice significantly increases the blood ethanol concentrations compared to non-dependent mice at the end of the drinking access period (Becker & Lopez, 2004; Dhaher et al., 2008; Finn et al., 2007). Additionally, greater ethanol consumption by the dependent mice also produces higher ethanol concentrations in the brain, reaching levels consistent with those produced during CIE exposure (Griffin, Lopez, Yanke, et al., 2009). Interestingly, the larger amount of ethanol consumed by the ethanol-dependent mice is not due to a longer drinking bout during the session, but rather it is due to a faster rate of drinking (i.e., more licks at the bottle) during the first 40 min of access compared to the non-dependent mice (Griffin, Lopez, Yanke, et al., 2009). The increase in voluntary drinking does not appear to be secondary to altered thirst states because water intake does not increase (Griffin, Lopez, Yanke, et al., 2009) and neither does it appear to be caused by the need for more calories because sucrose (Becker & Lopez, 2004) and saccharin (Lopez et al., 2012) intake are unaffected. Note, that in these studies, mice have free access to food and water at all times, even during vapor exposure. Importantly, the increased drinking phenotype by the dependent mice after CIE exposure has been replicated by several other laboratories (Contet et al., 2011; Depoy et al., 2013; Dhaher et al., 2008; Finn et al., 2007; Jeanes et al., 2011),

indicating that the increase in ethanol drinking gained by using this procedure is robust and reproducible.

The neurobiology of dependence-induced drinking

The neurobiological mechanisms driving increases in free-choice ethanol consumption are not completely understood, but are under active investigation by several laboratories using both mice and rats as model organisms. A comprehensive discussion is beyond the scope of this review, but there are some neuroadaptations contributing to increased drinking that parallel findings in humans and should briefly be considered. For example, up-regulation of glutamatergic activity after chronic ethanol treatment has been documented in a variety of animal models (Gass & Olive, 2008). In particular, microdialysis studies using rats have found elevated extracellular levels of glutamate following chronic ethanol treatment in the nucleus accumbens, dorsal striatum, and hippocampus (Dahchour & De Witte, 2003; Dahchour, Hoffman, Deitrich, & de Witte, 2000; Rossetti & Carboni, 1995). Similarly, magnetic resonance spectroscopy (MRS) studies in rats have shown increased glutamate activity in the prefrontal cortex (Hermann et al., 2012) and basal ganglia (Zahr et al., 2009). These findings are consistent with MRS studies in human alcoholics showing elevated glutamate activity in the anterior cingulate cortex and the nucleus accumbens (Bauer et al., 2013; Hermann et al., 2012). A recent report from our group found increased glutamatergic transmission in the nucleus accumbens of ethanol-dependent mice and also found that pharmacologically increasing the glutamate concentration further increased consumption (Griffin et al., 2013). Additionally, blockade of group II metabotropic glutamate receptors in the nucleus accumbens was significantly more effective at reducing drinking in ethanol-dependent mice than control mice (Griffin et al., 2013), consistent with the idea that increased glutamatergic tone in the nucleus accumbens contributed to the increased drinking by the dependent mice. Because the nucleus accumbens has long been implicated in reward processes (Ikemoto & Panksepp, 1999; Koob, 1998; Sesack & Grace, 2010), escalated free-choice drinking by the mice may be in part due to increased reinforcing efficacy of ethanol that is acquired during the establishment of ethanol dependence. Indeed, there is evidence that ethanol-dependent mice (Lopez, Anderson, & Becker, 2008) and rats (Valdez et al., 2002) have greater motivation to respond on levers for ethanol reinforcement.

On the other hand, negative reinforcement also plays an important role in elevated drinking during dependence and evidence indicates that alcoholics have a dysregulated stress response system (Ray, 2011; Tartter & Ray, 2012; von Bardeleben, Heuser, & Holsboer, 1989). These kinds of findings have fostered great interest in brain regions known to be involved in the stress response, particularly the amygdala and extended amygdala, where the corticotropin-releasing factor (CRF) system has been implicated in numerous preclinical reports as having a critical role in dependence-induced drinking in rodents. Several reviews on the topic are available (Koob & Le Moal, 2001, 2005, 2008). In particular, one of the CRF receptors (CRFR1) appears to be critical in dependence-induced ethanol drinking (Zorrilla, Heilig, de Wit, & Shaham, 2013). Studies in the rat model of dependence-induced drinking have found that these receptors are important in the excessive drinking by dependent rats compared to non-dependent rats (Valdez et al., 2002). In the mouse, similar findings have been observed. For example, microinjection of a CRF antagonist into the

amygdala blocks dependence-induced drinking (Finn et al., 2007) and deletion of the receptor by a knock-out strategy also blocks increased drinking by dependent mice (Chu et al., 2007). As a result of the preclinical work to understand the role of the CRF system in dependence, there has been an effort to develop synthetic ligands targeting the CRFR1 receptor for addiction and other psychiatric illnesses. A recent report reviewed some of the recent preclinical and clinical findings, and while challenges to developing these ligands as therapeutics were acknowledged, there are reasons to be optimistic about ongoing human clinical trials (Zorrilla et al., 2013).

Finally, some reports provide clues that other aberrant signaling mechanisms are also important for promoting the increased drinking in dependence. Although drinking mice were not used, Melendez et al. found transcriptional changes in the hippocampus, prefrontal cortex, and nucleus accumbens that were related to several different cellular processes during withdrawal after only 2 cycles of CIE exposure, e.g., brain-derived neurotrophic factor (BDNF) expression in the prefrontal cortex (Melendez et al., 2012). In another study that did use drinking mice, a variety of transcriptional changes were found in the extended amygdala of the dependent mice that substantiated increases in CRF mRNA found previously in dependent rats, and also identified other transcriptional changes not previously associated with ethanol dependence (Contet et al., 2011). Together, both of these studies indicate that ethanol dependence induces numerous transcriptional changes in several brain regions, highlighting the complex nature of ethanol dependence. Additional work is needed to determine whether these newly discovered transcriptional changes can be mechanistically linked to the excessive drinking by dependent mice.

Future considerations for CIE exposure and free-choice drinking

It should be possible to adapt CIE exposure procedures to other ethanol consumption models that also generate high rates of ethanol intake to determine if voluntary drinking can be increased even further. One possibility might be combining vapor inhalation procedures with the popular drinking-in-the-dark procedure that models binge drinking (Rhodes, Best, Belknap, Finn, & Crabbe, 2005; Rhodes et al., 2007), in which one session out of every 4 access sessions is 4 h in duration, leading to a large increase in ethanol intake. Additionally, given the recent demonstration that CIE exposure procedures could increase drinking in a continuous access model (Depoy et al., 2013), the CIE exposure procedures might be used in combination with the 24-h intermittent access model that has been shown to produce an escalation of intake in mice (Hwa et al., 2011; Hwa, Kalinichev, Haddouk, Poli, & Miczek, 2013; Melendez, 2011). Another intriguing alternative might be the use of several ethanol solutions in the same session, providing multiple choices for the mice. Some reports indicate that providing rodents with access to multiple bottles of different ethanol concentrations produces high levels of ethanol intake (Obara et al., 2009; Rodd et al., 2009; Rodd-Henricks et al., 2001). In any of these cases, it would be interesting to see if rendering mice ethanol-dependent using CIE exposure procedures increases voluntary consumption even further in these models or, in the case of making multiple ethanol concentrations available, causes a shift in preference toward solutions with higher concentrations of ethanol.

Laboratory mice are a great asset in biomedical research because of the genetic diversity that is associated with a varying propensity to drink ethanol. It has long been appreciated that different strains of mice have different preferences for ethanol (Belknap et al., 1993; Rodgers, 1972; Rodgers & McClearn, 1964), and current research is leveraging this diversity to understand the genetic determinants of ethanol drinking in models of ethanol dependence. Two important examples are the use of the inbred BXD strains of mice and the HAP/LAP study, both mentioned above. Although only several mouse strains were tested, the HAP/LAP study found that increases in ethanol consumption in the vapor inhalation model may be more readily observed in strains of mice with a high natural preference for ethanol and generally lower withdrawal seizure susceptibility, e.g., the B6 or the HAP-2 line (Lopez et al., 2011). The implication of these findings is that establishing ethanol dependence in a mouse strain that is relatively non-preferring does not necessarily lead to increased ethanol intake or preference. Clearly, this idea could be examined further using mice from the variety of BXD recombinant inbred strains or additional strains with varying propensities to consume ethanol.

In addition to the variety of mouse strains produced by selective breeding, there are also a tremendous variety of genetically modified mice available in which specific proteins are targeted, either knocked out or overexpressed, constitutively or conditionally, that could be used in the free-choice drinking and CIE model. For example, some data have already been published in the context of ethanol exposure using a NR2B subunit conditional knockout mouse (Badanich et al., 2011; Wills et al., 2012), and work is underway using these unique mice in the context of free-choice drinking and CIE exposure procedures. Data have already been published using CRFR1 knock-out mice showing that deletion of this receptor prevents escalation of ethanol self-administration (Chu et al., 2007). In the future, the use of mouse strains with specific targeted mutations is expected to grow and help unravel the complexity of neuroadaptations that produce increases in voluntary ethanol consumption.

Another consideration is the use of the free-choice drinking and CIE exposure model to investigate therapeutics that might be useful for reducing drinking in alcoholics. For example, topiramate, which demonstrates efficacy in treating alcoholics (Johnson et al., 2003), has been shown to reduce free-choice drinking by dependent mice in a preliminary report (Becker et al., 2013). Therefore, the preclinical model yields effects consistent with clinical experience, offering validation for the use of CIE exposure and free-choice drinking to test therapeutics in development for the treatment of alcoholism. Additionally, reports have shown that drugs targeting corticotropin-releasing factor transmission also reduce alcohol intake in mice (Finn et al., 2007) and rats (Gilpin et al., 2008), providing important guidance to clinical investigations (Zorrilla et al., 2013).

Conclusion

In the mouse model of free-choice drinking and ethanol dependence, consumption of the ethanol solution pre- and post-ethanol dependence is voluntary and offered as a free choice with water. As reviewed above, the CIE exposure using vapor inhalation procedures ultimately drives a substantial increase in voluntary ethanol drinking that is associated with increases in the rate of drinking during the session as well as higher blood and brain ethanol

concentrations in dependent mice compared to non-dependent controls. Importantly, the increased ethanol consumption in these procedures is specific to ethanol and unrelated to poor hydration status or increased caloric need. Additionally, the basic model of dependence and free-choice drinking offers some similarities to human alcoholic drinking which includes increased consumption, aberrant excitatory neurotransmission, and a dysregulated CRF transmission system. Further, there are data indicating that the excessive drinking in this model is amenable to treatment shown to be effective at reducing drinking in alcoholics. In conclusion, the mouse CIE exposure and free-choice drinking model offers a strong translational approach to our understanding of the neurobiological mechanisms that underlie human alcoholic drinking and may point the way to effective treatments for alcoholism.

Acknowledgments

WCG was supported by NIAAA P50 AA010761, U01 AA014095 and RO1 AA018036.

References

Badanich KA, Doremus-Fitzwater TL, Mulholland PJ, Randall PK, Delpire E, Becker HC. NR2B-deficient mice are more sensitive to the locomotor stimulant and depressant effects of ethanol. Genes, Brain, and Behavior. 2011; 10:805–816.

Bauer J, Pedersen A, Scherbaum N, Bening J, Patschke J, Kugel H, et al. Craving in alcohol-dependent patients after detoxification is related to glutamatergic dysfunction in the nucleus accumbens and the anterior cingulate cortex. Neuropsychopharmacology. 2013; 38:1401–1408. [PubMed: 23403696]

Becker H, Lopez M, Griffin W, Brubacher K, Johnson C, McCann R. Topiramate decreases voluntary intake in mouse models of excessive drinking and reduces expression of conditioned ethanol reward. Alcoholism: Clinical and Experimental Research. 2013; 37A:177A.

Becker HC. Animal models of excessive alcohol consumption in rodents. Current Topics in Behavioral Neurosciences. 2013; 13:355–377. [PubMed: 22371267]

Becker HC, Hale RL. Repeated episodes of ethanol withdrawal potentiate the severity of subsequent withdrawal seizures: an animal model of alcohol withdrawal “kindling”. Alcoholism: Clinical and Experimental Research. 1993; 17:94–98.

Becker HC, Lopez MF. Increased ethanol drinking after repeated chronic ethanol exposure and withdrawal experience in C57BL/6 mice. Alcoholism: Clinical and Experimental Research. 2004; 28:1829–1838.

Belknap JK, Crabbe JC, Young ER. Voluntary consumption of ethanol in 15 inbred mouse strains. Psychopharmacology. 1993; 112:503–510. [PubMed: 7871064]

Budygin EA, Oleson EB, Mathews TA, Läck AK, Diaz MR, McCool BA, et al. Effects of chronic alcohol exposure on dopamine uptake in rat nucleus accumbens and caudate putamen. Psychopharmacology. 2007; 193:495–501. [PubMed: 17492432]

Carrara-Nascimento PF, Lopez MF, Becker HC, Olive MF, Camarini R. Similar ethanol drinking in adolescent and adult C57BL/6J mice after chronic ethanol exposure and withdrawal. Alcoholism: Clinical and Experimental Research. 2013; 37:961–968.

Chu K, Koob GF, Cole M, Zorrilla EP, Roberts AJ. Dependence-induced increases in ethanol self-administration in mice are blocked by the CRF1 receptor antagonist antalarmin and by CRF1 receptor knockout. Pharmacology, Biochemistry, and Behavior. 2007; 86:813–821.

Contet C, Gardon O, Filliol D, Becker JA, Koob GF, Kieffer BL. Identification of genes regulated in the mouse extended amygdala by excessive ethanol drinking associated with dependence. Addiction Biology. 2011; 16:615–619. [PubMed: 21392173]

Dahchour A, De Witte P. Excitatory and inhibitory amino acid changes during repeated episodes of ethanol withdrawal: an in vivo microdialysis study. European Journal of Pharmacology. 2003; 459:171–178. [PubMed: 12524143]

Dahchour A, Hoffman A, Deitrich R, de Witte P. Effects of ethanol on extracellular amino acid levels in high-and low-alcohol sensitive rats: a microdialysis study. Alcohol and Alcoholism. 2000; 35:548–553. [PubMed: 11093960]

DeCarli LM, Lieber CS. Fatty liver in the rat after prolonged intake of ethanol with a nutritionally adequate new liquid diet. The Journal of Nutrition. 1967; 91:331–336. [PubMed: 6021815]

Depoy L, Daut R, Brigman JL, Macpherson K, Crowley N, Gunduz-Cinar O, et al. Chronic alcohol produces neuroadaptations to prime dorsal striatal learning. Proceedings of the National Academy of Sciences of the United States of America. 2013; 110:14783–14788. [PubMed: 23959891]

Dhaher R, Finn D, Snelling C, Hitzemann R. Lesions of the extended amygdala in C57BL/6J mice do not block the intermittent ethanol vapor-induced increase in ethanol consumption. Alcoholism: Clinical and Experimental Research. 2008; 32:197–208.

Fidler TL, Clews TW, Cunningham CL. Reestablishing an intragastric ethanol self-infusion model in rats. Alcoholism: Clinical and Experimental Research. 2006; 30:414–428.

Fidler TL, Dion AM, Powers MS, Ramirez JJ, Mulgrew JA, Smitasin PJ, et al. Intragastric self-infusion of ethanol in high- and low-drinking mouse genotypes after passive ethanol exposure. Genes, Brain, and Behavior. 2011; 10:264–275.

Fidler TL, Oberlin BG, Struthers AM, Cunningham CL. Schedule of passive ethanol exposure affects subsequent intragastric ethanol self-infusion. Alcoholism: Clinical and Experimental Research. 2009; 33:1909–1923.

Fidler TL, Powers MS, Ramirez JJ, Crane A, Mulgrew J, Smitasin P, et al. Dependence induced increases in intragastric alcohol consumption in mice. Addiction Biology. 2012; 17:13–32. [PubMed: 21955048]

Finn DA, Snelling C, Fretwell AM, Tanchuck MA, Underwood L, Cole M, et al. Increased drinking during withdrawal from intermittent ethanol exposure is blocked by the CRF receptor antagonist d-Phe-CRF(12-41). Alcoholism: Clinical and Experimental Research. 2007; 31:939–949.

Ford MM, Nickel JD, Finn DA. Treatment with and withdrawal from finasteride alter ethanol intake patterns in male C57BL/6J mice: potential role of endogenous neurosteroids? Alcohol. 2005; 37:23–33. [PubMed: 16472716]

Ford MM, Nickel JD, Phillips TJ, Finn DA. Neurosteroid modulators of GABA(A) receptors differentially modulate Ethanol intake patterns in male C57BL/6J mice. Alcoholism: Clinical and Experimental Research. 2005; 29:1630–1640.

Gass JT, Olive MF. Glutamatergic substrates of drug addiction and alcoholism. Biochemical Pharmacology. 2008; 75:218–265. [PubMed: 17706608]

Gilpin NW, Richardson HN, Koob GF. Effects of CRF1-receptor and opioid-receptor antagonists on dependence-induced increases in alcohol drinking by alcohol-preferring (P) rats. Alcoholism: Clinical and Experimental Research. 2008; 32:1535–1542.

Gilpin NW, Smith AD, Cole M, Weiss F, Koob GF, Richardson HN. Operant behavior and alcohol levels in blood and brain of alcohol-dependent rats. Alcoholism: Clinical and Experimental Research. 2009; 33:2113–2123.

Goldstein DB, Pal N. Alcohol dependence produced in mice by inhalation of ethanol: grading the withdrawal reaction. Science. 1971; 172:288–290. [PubMed: 5102255]

Grant BF, Dawson DA, Stinson FS, Chou SP, Dufour MC, Pickering RP. The 12-month prevalence and trends in DSM-IV alcohol abuse and dependence: United States, 1991-1992 and 2001-2002. Drug and Alcohol Dependence. 2004; 74:223–234. [PubMed: 15194200]

Green AS, Grahame NJ. Ethanol drinking in rodents: is free-choice drinking related to the reinforcing effects of ethanol? Alcohol. 2008; 42:1–11. [PubMed: 18164576]

Griffin WC 3rd, Lopez MF, Becker HC. Intensity and duration of chronic ethanol exposure is critical for subsequent escalation of voluntary ethanol drinking in mice. Alcoholism: Clinical and Experimental Research. 2009; 33:1893–1900.

Griffin WC 3rd, Lopez MF, Yanke AB, Middaugh LD, Becker HC. Repeated cycles of chronic intermittent ethanol exposure in mice increases voluntary ethanol drinking and ethanol concentrations in the nucleus accumbens. Psychopharmacology. 2009; 201:569–580. [PubMed: 18791704]

Griffin WC 3rd, Middaugh LD, Becker HC. Voluntary ethanol drinking in mice and ethanol concentrations in the nucleus accumbens. Brain Research. 2007; 1138:208–213. [PubMed: 17275791]

Griffin WC 3rd, Nguyen SA, Deleon CP, Middaugh LD. Effects of vigabatrin, an irreversible GABA transaminase inhibitor, on ethanol reinforcement and ethanol discriminative stimuli in mice. Behavioural Pharmacology. 2012; 23:178–190. [PubMed: 22336593]

Griffin WC III, Haun HL, Hazelbaker CL, Ramachandra VS, Becker HC. Increased Extracellular Glutamate In the Nucleus Accumbens Promotes Excessive Ethanol Drinking in Ethanol Dependent Mice. Neuropsychopharmacology. 2013 Epub ahead of print.

Hansson AC, Cippitelli A, Sommer WH, Ciccocioppo R, Heilig M. Region-specific down-regulation of Crhr1 gene expression in alcohol-preferring msP rats following ad lib access to alcohol. Addiction Biology. 2007; 12:30–34. [PubMed: 17407495]

Hansson AC, Rimondini R, Neznanova O, Sommer WH, Heilig M. Neuroplasticity in brain reward circuitry following a history of ethanol dependence. The European Journal of Neuroscience. 2008; 27:1912–1922. [PubMed: 18412612]

Healey JC, Winder DG, Kash TL. Chronic ethanol exposure leads to divergent control of dopaminergic synapses in distinct target regions. Alcohol. 2008; 42:179–190. [PubMed: 18358675]

Hermann D, Weber-Fahr W, Sartorius A, Hoerst M, Frischknecht U, Tunc-Skarka N, et al. Translational magnetic resonance spectroscopy reveals excessive central glutamate levels during alcohol withdrawal in humans and rats. Biological Psychiatry. 2012; 71:1015–1021. [PubMed: 21907974]

Holmes A, Fitzgerald PJ, MacPherson KP, DeBrouse L, Colacicco G, Flynn SM, et al. Chronic alcohol remodels prefrontal neurons and disrupts NMDAR-mediated fear extinction encoding. Nature Neuroscience. 2012; 15:1359–1361.

Hwa LS, Chu A, Levinson SA, Kayyali TM, DeBold JF, Miczek KA. Persistent escalation of alcohol drinking in C57BL/6J mice with intermittent access to 20% ethanol. Alcoholism: Clinical and Experimental Research. 2011; 35:1938–1947.

Hwa LS, Kalinichev M, Haddouk H, Poli S, Miczek KA. Reduction of excessive alcohol drinking by a novel GABAB receptor positive allosteric modulator ADX71441 in mice. Psychopharmacology. 2013 Epub ahead of print.

Ikemoto S, Panksepp J. The role of nucleus accumbens dopamine in motivated behavior: a unifying interpretation with special reference to reward-seeking. Brain Research. Brain Research Reviews. 1999; 31:6–41. [PubMed: 10611493]

Jeanes ZM, Buske TR, Morrisett RA. In vivo chronic intermittent ethanol exposure reverses the polarity of synaptic plasticity in the nucleus accumbens shell. The Journal of Pharmacology and Experimental Therapeutics. 2011; 336:155–164. [PubMed: 20947635]

Johnson BA, Ait-Daoud N, Bowden CL, DiClemente CC, Roache JD, Lawson K, et al. Oral topiramate for treatment of alcohol dependence: a randomised controlled trial. Lancet. 2003; 361:1677–1685. [PubMed: 12767733]

Koob GF. Circuits, drugs, and drug addiction. Advances in Pharmacology. 1998; 42:978–982. [PubMed: 9328061]

Koob GF, Le Moal M. Drug addiction, dysregulation of reward, and allostasis. Neuropsychopharmacology. 2001; 24:97–129. [PubMed: 11120394]

Koob GF, Le Moal M. Plasticity of reward neurocircuitry and the ‘dark side’ of drug addiction. Nature Neuroscience. 2005; 8:1442–1444.

Koob GF, Le Moal M. Addiction and the brain antireward system. Annual Review of Psychology. 2008; 59:29–53.

Lopez MF, Anderson RI, Becker HC. Repeated cycles of chronic intermittent ethanol exposure increase both self-administration and the reinforcing value of ethanol in C57BL/6J mice. Alcoholism: Clinical and Experimental Research. 2008; 32A:163A.

Lopez MF, Becker HC. Effect of pattern and number of chronic ethanol exposures on subsequent voluntary ethanol intake in C57BL/6J mice. Psychopharmacology. 2005; 181:688–696. [PubMed: 16001125]

Lopez MF, Grahame NJ, Becker HC. Development of ethanol withdrawal-related sensitization and relapse drinking in mice selected for high- or low-ethanol preference. Alcoholism: Clinical and Experimental Research. 2011; 35:953–962.

Lopez MF, Griffin WC 3rd, Melendez RI, Becker HC. Repeated cycles of chronic intermittent ethanol exposure leads to the development of tolerance to aversive effects of ethanol in C57BL/6J mice. Alcoholism: Clinical and Experimental Research. 2012; 36:1180–1187.

Melendez RI. Intermittent (every-other-day) drinking induces rapid escalation of ethanol intake and preference in adolescent and adult C57BL/6J mice. Alcoholism: Clinical and Experimental Research. 2011; 35:652–658.

Melendez RI, McGinty JF, Kalivas PW, Becker HC. Brain region-specific gene expression changes after chronic intermittent ethanol exposure and early withdrawal in C57BL/6J mice. Addiction Biology. 2012; 17:351–364. [PubMed: 21812870]

Mokdad AH, Marks JS, Stroup DF, Gerberding JL. Actual causes of death in the United States, 2000. JAMA. 2004; 291:1238–1245. [PubMed: 15010446]

Molander A, Vengeliene V, Heilig M, Wurst W, Deussing JM, Spanagel R. Brain-specific inactivation of the Crhr1 gene inhibits post-dependent and stress-induced alcohol intake, but does not affect relapse-like drinking. Neuropsychopharmacology. 2012; 37:1047–1056. [PubMed: 22113086]

O'Dell LE, Roberts AJ, Smith RT, Koob GF. Enhanced alcohol self-administration after intermittent versus continuous alcohol vapor exposure. Alcoholism: Clinical and Experimental Research. 2004; 28:1676–1682.

Obara I, Bell RL, Goulding SP, Reyes CM, Larson LA, Ary AW, et al. Differential effects of chronic ethanol consumption and withdrawal on homer/glutamate receptor expression in subregions of the accumbens and amygdala of P rats. Alcoholism: Clinical and Experimental Research. 2009; 33:1924–1934.

Ray LA. Stress-induced and cue-induced craving for alcohol in heavy drinkers: Preliminary evidence of genetic moderation by the OPRM1 and CRH-BP genes. Alcoholism: Clinical and Experimental Research. 2011; 35:166–174.

Rhodes JS, Best K, Belknap JK, Finn DA, Crabbe JC. Evaluation of a simple model of ethanol drinking to intoxication in C57BL/6J mice. Physiology & Behavior. 2005; 84:53–63. [PubMed: 15642607]

Rhodes JS, Ford MM, Yu CH, Brown LL, Finn DA, Garland T Jr. et al. Mouse inbred strain differences in ethanol drinking to intoxication. Genes, Brain, and Behavior. 2007; 6:1–18.

Richardson HN, Lee SY, O'Dell LE, Koob GF, Rivier CL. Alcohol self-administration acutely stimulates the hypothalamic-pituitary-adrenal axis, but alcohol dependence leads to a dampened neuroendocrine state. The European Journal of Neuroscience. 2008; 28:1641–1653. [PubMed: 18979677]

Roberts AJ, Cole M, Koob GF. Intra-amygdala muscimol decreases operant ethanol self-administration in dependent rats. Alcoholism: Clinical and Experimental Research. 1996; 20:1289–1298.

Rodd ZA, Bell RL, Kuc KA, Murphy JM, Lumeng L, McBride WJ. Effects of concurrent access to multiple ethanol concentrations and repeated deprivations on alcohol intake of high-alcohol-drinking (HAD) rats. Addiction Biology. 2009; 14:152–164. [PubMed: 19076927]

Rodd-Henricks ZA, Bell RL, Kuc KA, Murphy JM, McBride WJ, Lumeng L, et al. Effects of concurrent access to multiple ethanol concentrations and repeated deprivations on alcohol intake of alcohol-preferring rats. Alcoholism: Clinical and Experimental Research. 2001; 25:1140–1150.

Rodgers, DA. Factors underlying differences in alcohol preference of inbred strains of mice. In: Kissin, B.; Begleiter, H., editors. The Biology of Alcoholism. Plenum Press; New York: 1972. p. 107-130.

Rodgers DA, McClearn GE. Sucrose Versus Ethanol Appetite in Inbred Strains of Mice. Quarterly Journal of Studies on Alcohol. 1964; 25:26–35. [PubMed: 14128137]

Rossetti ZL, Carboni S. Ethanol withdrawal is associated with increased extracellular glutamate in the rat striatum. European Journal of Pharmacology. 1995; 283:177–183. [PubMed: 7498307]

Sesack SR, Grace AA. Cortico-Basal Ganglia reward network: microcircuitry. Neuropsychopharmacology. 2010; 35:27–47. [PubMed: 19675534]

Sharpe AL, Samson HH. Ethanol and sucrose self-administration components: effects of drinking history. Alcohol. 2003; 29:31–38. [PubMed: 12657374]

Sommer WH, Rimondini R, Hansson AC, Hipskind PA, Gehlert DR, Barr CS, et al. Upregulation of voluntary alcohol intake, behavioral sensitivity to stress, and amygdala crhr1 expression following a history of dependence. Biological Psychiatry. 2008; 63:139–145. [PubMed: 17585886]

Tartter MA, Ray LA. A prospective study of stress and alcohol craving in heavy drinkers. Pharmacology, Biochemistry, and Behavior. 2012; 101:625–631.

Valdez GR, Roberts AJ, Chan K, Davis H, Brennan M, Zorrilla EP, et al. Increased ethanol self-administration and anxiety-like behavior during acute ethanol withdrawal and protracted abstinence: regulation by corticotropin-releasing factor. Alcoholism: Clinical and Experimental Research. 2002; 26:1494–1501.

von Bardeleben U, Heuser I, Holsboer F. Human CRH stimulation response during acute withdrawal and after medium-term abstention from alcohol abuse. Psychoneuroendocrinology. 1989; 14:441–449. [PubMed: 2560222]

Wills TA, Klug JR, Silberman Y, Baucum AJ, Weitlauf C, Colbran RJ, et al. GluN2B subunit deletion reveals key role in acute and chronic ethanol sensitivity of glutamate synapses in bed nucleus of the stria terminalis. Proceedings of the National Academy of Sciences of the United States of America. 2012; 109:E278–287. [PubMed: 22219357]

Zahr NM, Mayer D, Vinco S, Orduna J, Luong R, Sullivan EV, et al. In vivo evidence for alcohol-induced neurochemical changes in rat brain without protracted withdrawal, pronounced thiamine deficiency, or severe liver damage. Neuropsychopharmacology. 2009; 34:1427–1442. [PubMed: 18704091]

Zorrilla EP, Heilig M, de Wit H, Shaham Y. Behavioral, biological, and chemical perspectives on targeting CRF(1) receptor antagonists to treat alcoholism. Drug and Alcohol Dependence. 2013; 128:175–186. [PubMed: 23294766]