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# 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.



## 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) (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

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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 to 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