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Operant Ethanol Self-Administration in Ethanol Dependent Mice

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Abstract

While rats have been predominantly used to study operant ethanol self-administration behavior in the context of dependence, several studies have employed operant conditioning procedures to examine changes in ethanol self-administration behavior as a function of chronic ethanol exposure and withdrawal experience in mice. This review highlights some of the advantages of using operant conditioning procedures for examining the motivational effects of ethanol in animals with a history of dependence. As reported in rats, studies using various operant conditioning procedures in mice have demonstrated significant escalation of ethanol self-administration behavior in mice rendered dependent via forced chronic ethanol exposure in comparison to nondependent mice. This paper also presents a summary of these findings, as well as suggestions for future studies.

Keywords

operant conditioning; ethanol self-administration; ethanol dependence; mice

Introduction

Ethanol self-administration using operant conditioning procedures has been firmly established in a number of species, including monkeys, rats, and mice (Meisch & Stewart, 1994; Samson, 1986). In these studies, animals are typically trained to make a response (e.g., press a lever) under a particular schedule of reinforcement, such that responding after either a specified number of responses (ratio schedules) or a specified period of time has elapsed (interval schedules) will result in delivery of ethanol as a reinforcer. In most studies involving oral ethanol self-administration, once the specified schedule of reinforcement was satisfied, the reinforcer (specific amount of an ethanol solution) was presented to the

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animals for consumption. In contrast to this procedure (termed the ‘dipper’ model), in other studies, once the response requirement was met, animals were provided free access to a bottle containing ethanol for a specified period of time (termed the ‘sipper’ model) (Samson, 2000).

It is well established that ethanol can serve as an effective positive reinforcer in these self-administration models. More recently, studies in animals with a history of dependence (chronic but ‘forced’ ethanol exposure and withdrawal) have demonstrated that ethanol can serve as a potent negative reinforcer as well. For example, increased ethanol self-administration was shown in studies where dependence was induced by chronic administration of ethanol in a nutritionally fortified liquid diet (that served as the animals’ sole source of calories and fluid) (Brown, Jackson, & Stephens, 1998; Chu, Koob, Cole, Zorilla, & Roberts, 2007; Gilpin et al., 2009; Schulteis, Hyytiä, Heinrichs, & Koob, 1996), via intragastric infusions (Cunningham, Fidler, Murphy, Mulgrew, & Smitasin, 2013; Fidler et al., 2011; Fidler et al., 2012), and via inhalation of alcohol vapors (e.g., Becker & Lopez, 2004; Rimondini, Arlinde, Sommer, & Heilig, 2002; Roberts, Heyser, Cole, Griffin, & Koob, 2000). In such studies, the altered physiological state associated with dependence along with the capacity for ethanol to alleviate withdrawal symptoms is posited to not only sustain ethanol self-administration, but also promote escalation of intake (Becker, 2008; Becker, 2013; Heilig, Egli, Crabbe, & Becker, 2010).

The use of operant conditioning procedures to study ethanol self-administration behavior has several important advantages over free-choice drinking models. First, this approach enables separate analysis of the appetitive (seeking) and consummatory (drinking) components of self-administration behavior. While the amount of ethanol consumed is a dependent variable common to all models of self-administration, studying the appetitive component provides an opportunity to examine the motivational effects of ethanol (i.e., how hard subjects will work to obtain access to ethanol). In addition, systematic manipulation of dose (e.g., ethanol concentration) as well as the schedule of reinforcement (i.e., increasing the response requirement using progressive ratio procedures) enables a more detailed analysis of the reinforcing efficacy of ethanol. Tracking the distribution and pattern of responding also provides a more refined analysis of factors that influence self-administration behavior. Additionally, measuring the behavioral response when ‘expected’ ethanol delivery is terminated (extinction responding) provides a means to operationally define ‘ethanol-seeking’ behavior. This procedure has been extensively used in relapse models to study how presentation of discrete conditioned cues (stimuli previously associated with ethanol reinforcement) and discriminative cues (context stimuli previously associated with occasions to self-administer ethanol) reinvigorate or reinstate ethanol responding that was experimentally extinguished. While all of these operant conditioning procedures have been predominantly used to study ethanol self-administration in rats, many of these procedures also have been adopted in studies with mice.

Operant Ethanol Self-Administration in Mice

Standard operant conditioning procedures have been employed to study ethanol self-administration behavior in mice under a variety of conditions. As in the case for rats, studies

have shown mice to respond for ethanol as a positive reinforcer when it is delivered orally (Meisch, 2001), intravenously (Grahame & Cunningham, 1997), and directly into the stomach via intragastric infusion (Fidler et al., 2011). While early studies showed that ethanol responding and intake is enhanced in food-deprived mice (Middaugh & Kelley, 1999), ethanol was demonstrated to be an effective positive reinforcer in non-food deprived mice as well (Ford, Fretwell, Mark, & Finn, 2007; Middaugh, Lee, & Bandy, 2000). Studies also have shown mice responding for ethanol when ethanol reinforcement is continuously available (Risinger, Brown, Doan, & Oakes, 1998), when it is made available for extended periods (~16 h) (Besheer, Lepoutre, & Hodge, 2004; Hodge et al., 2006), and when access is limited for short periods of time (~30–60 min) (Chu et al., 2007; Lopez, Anderson, & Becker, 2008; Lopez & Becker, 2014; Ramaker, Strong, Ford, & Finn, 2012; Sparta et al., 2009; Tsiang & Janak, 2006). Using the 'sipper' model described above, mice were shown to reliably respond to gain access to drink ethanol from a bottle made available for 30 min once the response requirement was satisfied (Finn et al., 2008; Ford et al., 2007). In this latter case, manipulating the reinforcement schedule to further separate the appetitive and consummatory components of the procedure enhanced both the appetitive drive to gain access to ethanol (as indicated by reduced latency to fulfill the response requirement) and the consummatory component (increased amount of ethanol consumed). Finally, a valuable feature of using mice in these studies is that it more readily facilitates examination of genetic contributions to operant ethanol self-administration behavior. Indeed, several studies have examined ethanol self-administration involving operant conditioning procedures in various genetic mouse models, including different inbred strains (Fidler et al., 2011; Grahame & Cunningham, 1997; Risinger et al., 1998), mice selectively bred for other ethanol-related phenotypes (Ford et al., 2011), and several genetically manipulated models engineered to be deficient in various target proteins (knockout models) (Grahame, Low, & Cunningham, 1998; Olive, Mehmert, Messing, & Hodge, 2000; Risinger, Doan, & Vickrey, 1999; Roberts et al., 2001; Roberts, McDonald, et al., 2000). Thus, while rats have been the predominant choice of species for operant ethanol self-administration studies, a growing body of literature indicates that operant conditioning procedures can be effectively employed in studying ethanol self-administration behavior in mice.

Operant Ethanol Self-Administration in Dependent Mice

As reviewed elsewhere (Becker, 2013), numerous studies utilizing operant conditioning procedures have demonstrated increased ethanol self-administration in rats following a history of chronic ethanol exposure and withdrawal experience (Funk & Koob, 2007; Funk, O'Dell, Crawford, & Koob, 2006; Funk, Zorilla, Lee, Rice, & Koob, 2007; Gilpin et al., 2009; O'Dell, Roberts, Smith, & Koob, 2004; Rimondini, Thorsell, & Heilig, 2005; Roberts, Cole, & Koob, 1996; Roberts, Heyser, et al., 2000). In contrast, only a handful of studies have been devoted to evaluating the effect of ethanol dependence on operant ethanol self-administration using mice. The following is a more detailed description of results generated from these studies.

In one study, male C57BL/6J mice were trained to respond for ethanol for several weeks (FR4, 10% ethanol; 60-min daily sessions). Once stable responding for ethanol was attained, half the mice received chronic intermittent exposure to ethanol vapors in inhalation

chambers (14 h/day for 21 days) and were given the opportunity to self-administer ethanol 8 h after being removed from the inhalation chambers each day. The self-administration sessions were extended for an additional 2 weeks after the chronic intermittent ethanol (CIE) vapor exposure was terminated. This study design resulted in elevated responding and a higher number of ethanol reinforcers earned for mice that experienced CIE exposure, but the effect was observed only during the 2 weeks after CIE exposure was terminated (Chu et al., 2007). During the 3 weeks of CIE exposure, ethanol self-administration was very similar to baseline levels and similar to control mice that did not receive ethanol vapor exposure (Chu et al., 2007). This profile of results differs from that reported in rats where ethanol self-administration was shown to progressively increase when the opportunity to respond for ethanol was provided during repeated acute withdrawal periods (Roberts et al., 1996; Roberts, Heyser, et al., 2000). This may reflect an important species difference in that mice may require a longer ‘recovery’ period following CIE exposure before being offered the opportunity to consume ethanol. It has been suggested that mice may require at least 48 h before ethanol is re-introduced to avoid potential conditioned taste aversion related to the CIE vapor exposure (Lopez & Becker, unpublished data).

In this study, mice were given the option to respond on one lever to obtain ethanol and on another lever to obtain water. Because of this feature, it was possible to observe that although the number of responses for ethanol reinforcement did not increase during the 3 weeks of CIE vapor exposure, preference for responding on the ethanol-related lever significantly increased in dependent mice during the course of CIE exposure, an effect that persisted after the chronic ethanol vapor exposure stopped (Chu et al., 2007). Another goal of this study was to examine whether genetic deletion of CRF1 receptors alters operant ethanol self-administration in dependent versus nondependent mice. In this case, baseline ethanol responding was first established and then wild-type controls (C57BL/6J X 129SvJ background) and CRF1 receptor knockout mice were exposed to ethanol delivered in a nutritionally fortified liquid diet for a 2-week period before operant testing resumed. Results indicated that while ethanol responding and preference were similar for both genotypes during the baseline phase, ethanol self-administration significantly increased in the wild-type controls but not CRF1 receptor knockout mice following the chronic ethanol treatment regimen (Chu et al., 2007). Thus, it was suggested that CRF1 receptors might play a significant role in mediating dependence-related escalation of ethanol self-administration.

Overall, these results are generally congruent with findings from a series of similar experiments conducted in our laboratory. Briefly, adult male C57BL/6J mice were trained to self-administer ethanol (FR4; 12% ethanol + 1% sucrose) in daily 15-min sessions. Once stable baseline ethanol responding and intake were established, one group of mice was exposed to CIE vapor exposure in inhalation chambers (16 h/day for 4 days) while the remaining mice were similarly handled but exposed to air in control inhalation chambers. After a forced abstinence period of 72 h, all mice were given the opportunity to self-administer ethanol in 5 daily operant sessions (as during baseline). This procedure was repeated for several cycles, with weekly CIE (or air) exposure cycles followed by a 72-h forced abstinence period and then 5-day self-administration sessions. Ethanol responding and intake were shown to significantly increase over baseline levels in dependent (CIE-exposed) mice as well as in comparison to nondependent controls, which remained relatively

stable throughout the experiment (Lopez & Becker, 2014). Importantly, increased ethanol self-administration in dependent mice (1.72 ± 0.18 g/kg) compared to nondependent mice (1.07 ± 0.10 g/kg) during the final test cycle resulted in significantly higher blood ethanol levels in CIE-exposed mice (128.1 ± 12.2 mg/dL) compared to controls (87.4 ± 12.2 mg/dL) registered immediately after the final 15-min operant session. Additionally, dependent (CIE-exposed) mice showed greater persistence in responding as the amount of work required to attain ethanol reinforcement was systematically increased (via progressive ratio schedules), increased resistance to extinction, and greater sensitivity to cue-induced reinstatement of ethanol responding in comparison to nondependent control mice (Lopez & Becker, 2014). Collectively, use of operant conditioning procedures in these studies (as opposed to free-drinking models) has provided valuable information regarding changes in the reinforcing efficacy of ethanol as well as relapse vulnerability as a function of a history of dependence.

Employing a different experimental strategy, Cunningham and colleagues used a model involving self-administration of ethanol delivered directly into the stomach. This model, first developed in rats (Deutsch & Koopmans, 1973; Fidler, Clews, & Cunningham, 2006), was recently adopted for mice. Although requiring labor-intensive surgical procedures, this model offers the advantage of bypassing orosensory cues associated with oral consumption of ethanol that could influence motivation to self-administer the drug. The procedure entails first exposing mice to a period of forced ethanol administration (3 infusions/day), followed by a 2-day period in which mice were given continuous access to a single bottle containing water that was artificially flavored. During this no-choice phase of the procedure, licks on the bottle (FR-10 schedule) resulted in delivery of an intragastric infusion of ethanol (10% concentration). This was followed by a 4-day choice phase in which mice were given a choice to drink from the bottle with a flavor that produces ethanol infusions or a second bottle with a different flavor that resulted in water infusions. Results indicated that forced intragastric infusions of ethanol resulted in increased self-administration of ethanol in both the no-choice and choice phases of the experiment (Fidler et al., 2011). Of particular interest, the study involved using this model to examine ethanol self-administration in mouse inbred strains that exhibit high (C57BL/6J) versus low (DBA/2J) ethanol preference, as well as mouse lines selectively bred for high (HAP2) versus low (LAP2) ethanol preference. Increased ethanol self-administration in the model was demonstrated in all mice, whether they typically exhibit high (C57BL/6J, HAP2) or low (DBA/2J, LAP2) ethanol intake under baseline conditions (Fidler et al., 2011). Thus, intragastric self-administration of ethanol was similar for all genotypes after a period of forced ethanol infusions even though these genotypes greatly differ in their acceptance of ethanol when it is presented for oral consumption. These results are also consistent with a previous study that showed similar levels of operant ethanol self-administration in C57BL/6J and DBA/2J inbred mice when ethanol was delivered via the intravenous route (Grahame & Cunningham, 1997).

In a series of follow-up experiments, Fidler et al. (2012) demonstrated that increased ethanol self-administration via intragastric infusions depends on the amount of ethanol infused and the duration of the forced infusion phase. Further, the increase in ethanol self-administration was proportional to the severity of withdrawal symptoms displayed by DBA/2J mice. This latter finding provides endorsement of the hypothesis that dependent mice may self-administer more ethanol to prevent and/or alleviate symptoms of withdrawal (Fidler et al.,

2012). This was further confirmed with studies in which the delay between the forced infusion phase and self-administration sessions was varied (Cunningham et al., 2013). Specifically, results indicated that increased ethanol self-administration was most robust in mice that had either no delay or a short (1-day) delay between the forced intragastric exposure phase and no-choice self-infusion phase, or between the no-choice and choice self-administration phases of the study. Furthermore, this effect was more pronounced in DBA/2J mice that are more sensitive to ethanol withdrawal than C57BL/6J mice (Cunningham et al., 2013). Taken together, results from this series of studies indicate that increased ethanol self-administration after a period of forced exposure may be driven, at least in part, by the negative reinforcing effects of ethanol in mice.

Conclusions and Future Directions

Although rats have been the species of choice in studies employing operant conditioning procedures to study ethanol self-administration in the context of dependence, a few studies have adopted similar procedures to evaluate changes in ethanol self-administration as a consequence of chronic ethanol exposure and withdrawal experience in mice. Since rodents (rats and mice) do not typically voluntarily consume sufficient amounts of ethanol to achieve a state of dependence, a number of procedures have been used to experimentally administer high doses of ethanol over a prolonged period of time to induce dependence. As reported in rats, studies in mice described in this review have demonstrated escalation of ethanol self-administration following chronic ethanol exposure delivered by inhalation (Chu et al., 2007; Lopez et al., 2008; Lopez & Becker, 2014), liquid diet (Chu et al., 2007), and intragastric infusion (Cunningham et al., 2013; Fidler et al., 2011; Fidler et al., 2012). These studies have taken advantage of employing operant conditioning procedures to enable a more refined analysis of ethanol consumption in dependent mice. For example, analysis of patterns of responding to gain access to ethanol provide information about 'drive' or 'motivation' for ethanol as well as frequency and size of ethanol bouts in dependent compared to nondependent mice (Fidler et al., 2012). In addition, ethanol-dependent mice were shown to develop a conditioned preference for a flavor associated with ethanol self-infusion after a chronic regimen of forced (passive intragastric) exposure, thereby enabling evaluation of ethanol’s reinforcing effects even though the drug was not being orally consumed (Fidler et al., 2011). Similarly, ethanol-dependent mice exhibited greater responding for ethanol even when the amount of work required to obtain ethanol reinforcement was progressively increased, persisted in responding even when ethanol reinforcement was no longer made available (extinction testing), and also showed enhanced cue-induced reinstatement of ethanol-seeking behavior while tested in the absence of ethanol availability (Lopez & Becker, 2014).

Although few in number, the studies presented here offer important information about factors that should be considered in evaluating operant ethanol self-administration in dependent mice. In some of the studies, mice were trained to self-administer ethanol prior to chronic ethanol exposure via inhalation (Chu et al., 2007; Lopez et al., 2008; Lopez & Becker, 2014) or liquid diet (Chu et al., 2007), and then were provided an opportunity to self-administer ethanol again (post-dependence). This experimental strategy is based on the notion that it would be optimal to first establish the positive reinforcing effects of ethanol

before the animals acquire the negative reinforcing effects of ethanol (Meisch, 1983). However, in other studies involving forced intragastric ethanol infusions, mice were given their first opportunity to self-administer ethanol under free-choice conditions after the chronic exposure regimen (Cunningham et al., 2013; Fidler et al., 2011; Fidler et al., 2012). This raises some questions about whether a baseline period of ethanol self-administration that presumably establishes the positive reinforcing effects of the drug is required before the negative reinforcing effects of ethanol can be acquired through repeated access to ethanol during chronic ethanol exposure and withdrawal cycles. It is unclear whether the mode of chronic ethanol treatment is a critical variable that influences this procedural difference. In this vein, the longer post-ingestive delay in reinforcement when the drug is consumed orally (as opposed to direct infusion into the stomach) may require a longer period of time to establish the positive reinforcing (and negative) reinforcing effects of ethanol. Of course, there is some model face validity in first establishing a baseline (moderate) level of ethanol consumption prior to evaluating the ability of chronic ethanol exposure and withdrawal experience to augment ethanol self-administration.

Another issue raised in these studies on operant ethanol self-administration behavior in dependent mice is the temporal relationship between the time when access to self-administer ethanol is given in relation to withdrawal from chronic ethanol treatment. In one study, escalation of operant ethanol self-administration was not apparent when it was offered 8 h into each withdrawal period following chronic ethanol vapor exposure (Chu et al., 2007). In fact, increased ethanol responding was not evident until after CIE exposure was terminated. This is similar to findings from our lab indicating that a 48–72 h delay may be optimal for observing increased ethanol self-administration following CIE vapor exposure (Lopez & Becker, unpublished findings). Together, these results suggest that a sufficient period of ‘recovery’ (> 8 h forced abstinence) following chronic ethanol exposure may be required to observe augmented self-administration. However, studies involving forced intragastric infusions indicated that increased ethanol self-administration was optimal when it coincided during acute withdrawal periods (Cunningham et al., 2013). Thus, this issue merits further investigation.

In summary, despite the limited number of studies conducted with mice using operant conditioning procedures, increased ethanol self-administration has been demonstrated following different chronic ethanol treatment regimens. Future studies using these models should continue to explore behavioral and environmental factors that influence propensity to self-administer ethanol in the context of dependence, as well as how a history of such dependence may alter vulnerability to relapse, as defined by operant reinstatement models. The use of mice in studying operant ethanol self-administration within the context of dependence can easily be adopted to investigate genetic factors that influence this behavior (through use of various genetic mouse models). Another area in which use of operant conditioning procedures will be valuable is in studies assessing the extent to which elevated ethanol self-administration behavior in dependent mice reflects a shift in bias from goal-directed (action-outcome) to habit-like (stimulus-response) processes. Finally, use of operant conditioning procedures can be used to examine risk factors (e.g., high impulsivity and anxiety traits) in conferring greater vulnerability to transition to dependence-related excessive ethanol self-administration as well as factors that contribute to compulsive-like

behavior that may drive higher levels of ethanol seeking and consumption in dependent mice.

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