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Operant alcohol self-administration in dependent rats: focus on the vapor model

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Abstract

Alcoholism (alcohol dependence) is characterized by a compulsion to seek and ingest alcohol (ethanol), loss of control over intake, and the emergence of a negative emotional state during withdrawal. Animal models are critical in promoting our knowledge of the neurobiological mechanisms underlying alcohol dependence. Here, we review the studies involving operant alcohol self-administration in rat models of alcohol dependence and withdrawal with the focus on the alcohol vapor model. In 1996, the first articles were published reporting that rats made dependent on alcohol by exposure to alcohol vapors displayed increased operant alcohol self-administration during acute withdrawal compared with nondependent rats (i.e., not exposed to alcohol vapors). Since then, it has been repeatedly demonstrated that this model reliably produces physical and motivational symptoms of alcohol dependence. The functional roles of various systems implicated in stress and reward, including opioids, dopamine, corticotropin-releasing factor (CRF), glucocorticoids, neuropeptide Y (NPY), γ -aminobutyric acid (GABA), norepinephrine, and cannabinoids, have been investigated in the context of alcohol dependence. The combination of models of alcohol withdrawal and dependence with operant self-administration constitutes an excellent tool to investigate the neurobiology of alcoholism. In fact, this work has helped lay the groundwork for several ongoing clinical trials for alcohol dependence. Advantages and limitations of this model are discussed, with an emphasis on what future directions of great importance could be.

Keywords

alcoholism; addiction; alcohol dependence; alcohol (ethanol) vapor; operant self-administration; compulsive behavior; rat; review

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Introduction

Increased operant alcohol (ethanol) self-administration in rats associated with alcohol dependence and withdrawal produced by alcohol vapor exposure was first demonstrated in 1996 (Roberts et al., 1996). However, there was a very important body of work published prior to this that was critical in the development of this rat/ethanol-vapor/operant model. This history will be summarized in a manner that will highlight aspects of this model that engender excessive alcohol intake. We will also review what has been discovered using the vapor/operant model with respect to both environmental and biological factors. Finally, we will discuss advantages and limitations of this model with an emphasis on what future directions we believe could be of great importance. But first, what was the motivation to develop such a model? Why drinking subsequent to dependence? Why operant self-administration? Why rats?

Alcohol was involved in 3.5% of deaths in the United States in 2000, making it the third-leading cause of preventable death in this country (Mokdad et al., 2004). Alcohol abusers drink perhaps partly for its euphorigenic effects, but progressively more in order to avoid or reverse the negative symptoms associated with withdrawal (Cappell & LeBlanc, 1981; Edwards, 1990). Indeed, the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (DSM-IV) criteria for substance dependence on alcohol include a withdrawal syndrome and taking the substance (or a closely related substance) to relieve or avoid withdrawal symptoms (American Psychiatric Association, 2000), similar to the DSM-V criteria for moderate to severe substance use disorder (O'Brien, 2011; Peer et al., 2013). The affective components of withdrawal, such as anxiety, dysphoria, and depressed mood, create a motivational drive that leads to compulsive ethanol drinking behavior and relapse even after long periods of abstinence (Hershon, 1977). These affective symptoms begin as blood alcohol levels drop and can continue for weeks to months to years following withdrawal (Alling et al., 1982; Mossberg et al., 1985; Parsons et al., 1990). Alcohol dependence is associated with high rates of relapse, which is characterized by a return to drinking after a period of abstinence and involves the consumption of excessive amounts of alcohol (U.S. Department of Health and Human Services, 1990). Therefore, alcohol dependence is a disorder with chronic relapses, with serious consequences to the individual, family, and society. Therefore, having a model of ethanol self-administration in animals experiencing withdrawal and in abstinent animals is important for the advancement of better prevention and treatment approaches.

Free-choice bottle drinking models capture consummatory aspects, whereas operant self-administration is more versatile in modeling different behavioral aspects of alcohol drinking. Both the appetitive/motivational (e.g., pressing a lever [workload] to receive a dose of alcohol) and consummatory (e.g., drinking the alcohol) components of ethanol consumption can be studied in operant models (Cunningham et al., 2000; Tabakoff & Hoffman, 2000). Appetitive behaviors become compulsive as dependence progresses (Koob, 2013), in that they become persistent and repetitive without leading to actual reward or pleasure. Compulsivity is described in the DSM: continued use despite knowledge of having had a persistent or recurrent physical or psychological problem and a great deal of time spent in activities necessary to obtain the substance. Thus, assessing operant ethanol self-

administration in dependent animals allows the appetitive and consummatory processes and, in particular, the compulsive nature of addiction to be studied.

Finally, there is a rich history of using rats in behavioral brain research. Issues of reliability and validity are critical in developing and utilizing animal models of complex neuropsychiatric disorders and must always be considered (Edwards & Koob, 2012; Geyer & Markou, 1995). As discussed by Bell and colleagues (2012), an effective animal model of alcoholism should include both positive (euphoric) and negative (eliminating negative aspects of withdrawal) reinforcement aspects. Several such models, including the one described in this review, have been developed, and are invaluable for studies of neuropharmacological mechanisms of alcoholism that would be impossible to do in humans. For example, significant understanding of the neurocircuitry involved in drug-seeking behavior in the addicted state has come from rat studies, and, indeed, rat neuropharmacological studies continue to drive the development of new medication targets (Koob, 2010).

History and Highlights in the Development of the Rat Operant/Vapor Model

Previous studies examined ethanol-drinking behavior in dependent animals. Both increases (Deutsch & Koopmans, 1973; Deutsch & Walton, 1977; Hunter et al., 1974; Samson & Falk, 1974; Schulteis et al., 1996; Veale & Myers, 1969; Wolffgramm & Heyne, 1991) and decreases (Begleiter, 1975; Myers et al., 1972; Winger, 1988) in ethanol intake were observed. In examining these studies, it became clear that there were two general concepts that likely played a role in these differential results that would require attention prior to being able to produce a robust and reliable model. These were issues of ethanol's palatability and reinforcing properties prior to induction of dependence and concerns regarding the post-dependence withdrawal spectrum. Specifically, how should researchers produce dependence while minimizing the potential of excessive physical symptoms that would compete with appetitive and/or consummatory behaviors, thus allowing the animals to learn the association between drinking ethanol and the alleviation of withdrawal symptoms?

Deutsch and Walton (1977) used a procedure in which the rats drank a flavored solution to receive infusions of ethanol directly into the stomach and showed that dependence enhanced preference for the ethanol-paired flavor. This model bypassed the aversive taste properties of ethanol and allowed ethanol to become a reinforcer. Historically, low levels of intake hampered rat models of oral ethanol self-administration unless the animals were food- or fluid-deprived. Consumption, therefore, could be motivated by thirst or the need for the calories in the ethanol solution, and not by ethanol's pharmacological effects. This changed with the breeding of ethanol-preferring rat strains (reviewed by McBride, this issue) and, in outbred strains, the development of the sweetened solution fading procedure by Samson and colleagues (Samson, 1986). In the latter model, ethanol is initially sweetened, and ethanol concentrations are gradually increased such that non-deprived rats will maintain lever pressing for high concentrations of ethanol that result in pharmacologically relevant blood alcohol levels. The sweetener is then removed gradually so that by the end of the procedure, the rats are drinking unsweetened alcohol solutions. This development paved the way for

subsequent studies by partially solving both the palatability and physiological need issues. Nonetheless, these procedures do not result in levels of alcohol intoxication to the point of dependence.

The problem of making the rats dependent was an outstanding issue. It was known that most rats would not voluntarily consume enough ethanol to induce dependence (Myers & Veale, 1972; Samson et al., 1988; Veale & Myers, 1969). Rats can be made dependent on alcohol by repeated exposure to high doses of alcohol via gastric intubation, oral gavage, mixing ethanol into a liquid diet, and systemic injections. While these techniques have been successfully used to produce dependence and subsequent increases in ethanol intake (Deutsch & Walton, 1977; Hunter et al., 1974; Schulteis et al., 1996), intubation or injections require either stressful repeated administration or surgical intragastric cannulation. Ethanol-containing liquid diet-induced dependence has been shown to produce escalated alcohol self-administration during acute withdrawal compared with control rats (Schulteis et al., 1996). However, this response pattern depends on high blood alcohol levels at the time of withdrawal, and intake during the dependence induction phase can be difficult to control in this model. In addition, the liquid diet approach can have the caveat of potential malnourishment in both the ethanol- and pair-fed groups (Rogers et al., 1979). Several laboratories began employing vaporized ethanol exposure to induce dependence (i.e., Karanian et al., 1986; Rigger et al., 1980; Rogers et al., 1979). This method has the benefit of more precise control of blood alcohol levels across varying periods of time and therefore allows for the examination of known exposure patterns on behavior, physiology, and biochemistry.

The second challenge with dependence is capitalizing on the affective symptoms without the excessive physical symptoms rendering the rats incapable of appetitive or consummatory behavior. In all three of the studies that showed decreased ethanol intake following dependence, significant physical withdrawal symptoms were observed. The majority of the rats in the Begleiter (1975) study had convulsions, and all of the monkeys in both the Myers et al. (1972) and Winger (1988) studies showed tremor during withdrawal.

Finally, a critical component of the DSM criteria for substance dependence on alcohol or moderate to severe substance use disorder must be established, namely taking the substance (or a closely related substance) to relieve or avoid withdrawal symptoms (American Psychiatric Association, 2000; O'Brien, 2011; Peer et al., 2013). This requires the animal to experience symptoms of withdrawal or abstinence with ethanol available and then to associate ethanol intake with the alleviation of these symptoms. Hunter and colleagues (1974) showed that rats did not voluntarily consume ethanol following a single 20-day period of forced liquid diet despite the presence of withdrawal symptoms. However, following a series of 3 liquid diet exposures followed by free choice testing, the rats transiently increased ethanol-drinking behavior, suggesting that the rats needed to learn the association between drinking ethanol and the alleviation of withdrawal symptoms.

Fig. 1 (Roberts et al., 1996) illustrates several of the aspects described above, that are believed to be critical in developing a successful model of increased operant ethanol self-administration in rats associated with alcohol dependence and withdrawal. First, operant

ethanol self-administration was initially established in non-deprived animals, such that lever pressing for 10% ethanol was stable. Second, operant responding for 10% ethanol was enhanced following dependence induction by 14 days of continuous ethanol vapor exposure, maintaining blood alcohol levels of 150 to 200 mg%. This blood alcohol level range is associated with mild-to-moderate withdrawal symptoms (Macey et al., 1996); therefore, it appeared that there was no interference in lever pressing and drinking behavior by physical symptoms. The rats were allowed access to ethanol immediately following removal from the vapor chambers for 12 h across several withdrawal periods. Therefore, the rats were given the opportunity to associate ethanol consumption with the alleviation (and apparently avoidance by the second test) of symptoms as they arose.

This model has evolved over the years to include operant ethanol self-administration testing after several weeks of withdrawal (Gilpin et al. 2008a, b; Roberts et al., 2000; Sommer et al., 2008), the use of intermittent ethanol vapor exposure to produce more rapid increases in the self-administration of ethanol relative to continuous exposure, and operant testing 6–8 h into withdrawal during these intermittent vapor exposure periods (O'Dell et al., 2004). The next section describes the rat/ethanol-vapor/operant model used currently and its use in elucidating neuropharmacological correlates of the increased operant ethanol self-administration following dependence induction. The focus of this review is on the alcohol vapor model, but a few studies involving liquid diet, which produces blood alcohol levels comparable to vapor exposure, will be discussed.

Significant Environmental and Biological Factors Associated with this Model

Operant self-administration is typically conducted in standard operant conditioning chambers fitted with retractable levers and fluid receptacles. As mentioned above, because of the aversive palatable properties of alcohol, rats will normally not readily press a lever to drink alcohol. To overcome this issue, a method based on that developed by Samson (1986) is used to facilitate the acquisition of alcohol self-administration. Briefly, rats are trained to press a lever to receive a highly palatable sweet solution (e.g., 10% sucrose). Alcohol is gradually added to the solution, and sucrose is gradually eliminated. At the end of the training (a few weeks), rats will lever press to receive an unsweetened alcohol solution. Some variations of this method have been described (Walker & Koob, 2007). Alternatively, rats are trained to lever press for water in long operant sessions (e.g., overnight). Subsequently, alcohol solution is provided instead of water in 2-h, 1-h, and then 30-min sessions (Edwards et al., 2012; Vendruscolo et al., 2012). Operant training is typically performed on a fixed-ratio 1 (FR1) schedule of reinforcement, in which every operant response is reinforced with the delivery of alcohol solution.

Upon the completion of operant self-administration training, the rats are exposed to continuous or intermittent alcohol vapor exposure (blood and brain alcohol levels between 150–250 mg/dL; Gilpin et al., 2009) to produce alcohol dependence (see Gilpin et al., 2008a, for review). Operant alcohol self-administration sessions are performed during acute (2–10 h into withdrawal from alcohol vapor) or protracted (one or more weeks after removal from vapor) withdrawal. In this model, rats exhibit physical withdrawal symptoms (e.g., tail

stiffness, abnormal gait/posture and tremor; Macey et al., 1996) and emotional symptoms, reflected by increased anxiety-like behavior, hyperalgesia, increased 22 kHz ultrasonic vocalizations, and elevated brain reward thresholds (Edwards et al., 2012; O'Dell et al., 2004; Rimondini et al., 2003; Roberts et al., 2000; Schulteis et al., 1995; Sommer et al., 2008; Valdez et al., 2002; Williams et al., 2012; Zhao et al., 2007). Nondependent rats are maintained and tested under similar operant conditions but are not exposed to alcohol vapors. Intermittent alcohol vapor exposure has been shown to produce a more rapid escalation of alcohol intake compared with continuous vapor exposure (O'Dell et al., 2004), indicating that repeated cycles of intoxication and withdrawal are more effective in producing dependence.

As illustrated in Fig. 2, rats exposed to chronic, intermittent alcohol vapor and tested during acute withdrawal (6–8 h after removal from vapor) display increased alcohol self-administration compared with nondependent rats in an FR1 schedule of reinforcement (Fig. 2A). Responding on an FR1 schedule requires minimal effort by the animal to obtain alcohol and is sometimes considered a consummatory measure. Dependent rats also display increased response levels for alcohol on a progressive-ratio (PR) schedule of reinforcement, under which the number of lever presses necessary to obtain the next availability of alcohol increases progressively (Fig. 2B). In this test, the workload (“price”) for the next alcohol reinforcer increases progressively until the rat reaches a “breakpoint” (i.e., a measure of compulsivity) beyond which it no longer performs for alcohol. Additionally, dependent rats display more persistent consumption of alcohol than nondependent rats as quinine concentrations that are added to the solution are increased (Fig. 2C; i.e., they maintain ethanol consumption despite the aversive bitter taste of quinine). This is considered another measure of compulsive-like alcohol intake. Finally, dependent rats did not differ from nondependent rats in relation to the self-administration of a sweet solution (without alcohol), indicating that the escalation of alcohol intake is specific for alcohol (Fig. 2D; O'Dell et al., 2004). These findings suggest that alcohol vapor exposure produces escalation of alcohol intake and compulsive-like drinking that is specific for alcohol (Vendruscolo et al., 2012).

The positive reinforcing properties of alcohol initially motivate alcohol intake. Alcohol self-administration increases the levels of dopamine and serotonin (5-HT) in the nucleus accumbens (NAc; Weiss et al., 1996). However, following a prolonged history of alcohol dependence, negative reinforcement becomes a dominant motivational factor for continued alcohol use (i.e., alcohol is used to alleviate or prevent negative emotional states, such as anxiety, dysphoria, and hypohedonia, that emerge in the absence of the drug). During the development of dependence, neurotransmission in reward-/stress-related brain regions is changed. In fact, in addition to changes in the systems involved in the initial reinforcing effects of ethanol, other systems are recruited as these negative motivational factors become critically important.

For example, the suppression of dopamine levels in the NAc occurs with chronic ethanol liquid diet exposure, and these levels are normalized after alcohol self-administration, suggesting that a deficit in dopamine release in the NAc may motivate dependent rats to more vigorously lever press for alcohol to normalize their dopamine levels (Weiss et al., 1996).

Differences in 5-HT and dopamine systems with respect to ethanol withdrawal and ethanol self-administration have also been reported in rats with left vs. right functional brain asymmetry. Similar to humans, rats also exhibit patterns of hemispheric specialization, and turning behavior is used as an index of the degree and direction of asymmetry. Animals with right turning preferences displayed lower 5-HT and dopamine concentrations in the right side of the prefrontal cortex compared with the left side during withdrawal from alcohol exposure via liquid diet. Animals with left turning preferences displayed opposite results with regard to dopamine concentrations in the prefrontal cortex during withdrawal. Interestingly, exposure to alcohol vapor increased alcohol self-administration in right-turners only, indicating that functional brain asymmetry and differences in dopamine and 5-HT neurotransmission modulate escalated alcohol intake (Carlson & Stevens, 2006).

A critical goal of pharmacological studies is to reverse the allostatic changes in the brain that occur during dependence. Allostasis, literally “stability with change,” is an alternative to homeostasis that allows dynamic adjustments to maintain stability in the face of predictable and unpredictable change. While allostasis is a critical mechanism, it requires more energy and is more subject to perturbation than homeostatic regulation, and therefore can be deleterious in the long run, leading to allostatic load/overload (McEwen & Stellar, 1993). The transition from controlled to excessive alcohol intake has been hypothesized to involve a change in reward set point caused by an allostatic process (Koob & Le Moal, 1997; Roberts et al., 2000). Conceptually, the neuroadaptive changes caused by chronic intermittent ethanol may contribute to allostatic load in the brain, thus disrupting reward circuitry as well as other brain functions (e.g., emotional regulation), physiological processes (e.g., body temperature regulation and sleep), and cognitive processes (Koob, 2008). Therefore, it is important to study systems that are recruited in this allostatic process.

An “ideal” treatment for alcoholism would decrease alcohol drinking in dependent individuals without changing alcohol drinking in nondependent individuals. The alcohol vapor/operant self-administration model possesses good predictive validity, such that acamprosate and naltrexone, two FDA-approved drugs to treat alcoholism, decrease alcohol self-administration in dependent rats (Morse & Koob, 2002; Walker & Koob, 2008). However, these drugs also decreased nondependent drinking, suggesting that they may be interfering with the positive reinforcing properties of alcohol. Investigations of other systems are therefore essential for the discovery of better drugs to effectively and specifically treat alcohol dependence. Table 1 summarizes the results of pharmacological manipulation of several systems (e.g., opioids, corticotropin-releasing factor [CRF], neuropeptide Y [NPY], γ -aminobutyric acid [GABA], norepinephrine, and cannabinoids) on operant self-administration in dependent and nondependent rats.

Systemic, acute administration of MPZP, a small-molecule, nonpeptide, brain-penetrant antagonist with high affinity for CRF₁ receptors, decreased alcohol self-administration in dependent rats (Richardson et al., 2008a). Injections of the CRF receptor antagonist D-Phe-CRF₁₂₋₄₁ into the cerebroventricular (CV) and central nucleus of the amygdala (CeA) also decreased alcohol self-administration in dependent rats, but did not change alcohol self-administration in nondependent rats; this effect was not seen in the bed nucleus of the stria terminalis (BNST) or NAc (Funk et al., 2006; Valdez et al., 2002). Repeated treatment

(every other day) with another CRF₁ receptor antagonist, R121919, reduced alcohol self-administration in both dependent and nondependent rats (Roberto et al., 2010). The CRF₂ receptor agonist urocortin 3, when acutely injected ICV (Valdez et al., 2004) or intra-CeA (Funk and Koob, 2007) reduced alcohol self-administration specifically in rats made dependent on alcohol via liquid diet or vapor exposure. These findings suggest that decreased CRF₁ receptor activity and increased CRF₂ receptor activity specifically reduce alcohol drinking in dependent rats.

Acute ethanol exposure activates the HPA axis to release CRF from the paraventricular nucleus of the hypothalamus (PVN), adrenocorticotrophic hormone (ACTH) from the pituitary, and corticosterone from the adrenal glands in the rat (Ellis, 1966; Richardson et al., 2008b). Chronic alcohol exposure appears to produce excessive activation of the HPA axis, ultimately leading to a dampening of HPA axis stimulation (Richardson et al., 2008b). These effects may be partially due to changes in CRF levels and sensitivity in the PVN and pituitary (Richardson et al., 2008b). Recently, it has been reported that corticosteroid-dependent plasticity is important in the escalated alcohol intake associated with dependence. Chronic glucocorticoid receptor (GR) antagonism by mifepristone blocked the enhancement of alcohol self-administration in vapor-exposed rats during acute withdrawal without altering nondependent drinking (Vendruscolo et al., 2012). These findings suggest that preventing corticosterone-induced excessive activation of GRs during alcohol intoxication and withdrawal via chronic GR blockade may prevent allostatic changes in brain stress systems and block escalated alcohol drinking.

Neuropeptide Y is a stress-related peptide that has been shown to participate in alcohol dependence. Thorsell et al. (2005) trained rats to press a lever 20 times to receive access to alcohol for 25 min. After alcohol vapor exposure, the rats were tested with a fixed time schedule in which access to alcohol was provided 10 min after the session began, regardless of the number of lever presses. This schedule allowed for the independent evaluation of both the motivation to obtain alcohol (lever presses) and alcohol intake. Dependent rats displayed increased alcohol intake, an effect that was blocked by acute ICV infusions of NPY. Interestingly, dependent rats did not show increased lever-pressing behavior using this paradigm. Using an FR1 schedule of reinforcement, Gilpin et al. (2011) reported that chronic/repeated ICV NPY infusions reduced alcohol self-administration in both dependent and nondependent rats. However, NPY Y₂ receptor antagonists acutely injected, systemically or intra-CeA, produced no effect on alcohol self-administration in dependent or nondependent rats (Kallupi et al., 2013). The actions that NPY has on alcohol dependence have been suggested to involve the modulation of GABA neurotransmission (Gilpin et al., 2011).

Acute intra-CeA injection of the potent, selective agonist for the GABA_A receptor muscimol decreased responding for alcohol specifically in dependent rats (Roberts et al., 1996). Other studies have investigated the role of GABA_B receptors on alcohol dependence. The GABA_B receptor agonist baclofen acutely decreased responding for alcohol in both dependent and nondependent rats in FR1 and PR schedules of reinforcement, with increased sensitivity in dependent rats (Walker & Koob, 2007). Gabapentin produces distinct electrophysiological changes in the CeA in dependent compared with nondependent rats via GABA_B receptors.

Consistent with these results, acute systemic injection of gabapentin reduced escalated alcohol self-administration in dependent rats, with no effect on nondependent drinking (reviewed by Clemens & Vendruscolo, 2008; Roberto et al., 2008). These results suggest that while NPY and GABA systems play an important role in alcohol dependence, they might also affect the positive reinforcing properties of alcohol.

The opioid system, especially the κ -opioid system, has been shown to play a specific role in alcohol dependence. Acute naltrexone, a preferential μ -opioid receptor antagonist, and nalmefene, a μ - and κ -opioid receptor antagonist, blocked alcohol self-administration in both dependent and nondependent rats (Kissler et al., 2013), with nalmefene being more effective than naloxone in reducing intake in dependent rats. Persistent κ -opioid receptor antagonism by nor-Binaltorphimine (nor-BNI) injected systemically (Walker & Koob, 2008; Walker et al., 2011), intra-NAc shell (Nealey et al., 2011), or intra-CeA reduced alcohol self-administration exclusively in dependent rats. Interestingly, the σ -opioid receptor antagonist BD-1063 dose-dependently reduced alcohol self-administration in dependent but not nondependent rats (Sabino et al., 2009).

Acute administration of the β -adrenergic receptor blocker propranolol but not nadolol, which does not cross the blood-brain barrier, reduced alcohol self-administration in dependent rats. A high dose of propranolol reduced responding for alcohol (FR1 and PR tests) in both dependent and nondependent rats (Gilpin & Koob, 2010). The α_1 -noradrenergic receptor antagonist prazosin acutely decreased alcohol self-administration in dependent and nondependent rats, with higher sensitivity in dependent rats (Walker et al., 2008).

Cannabinoid and vasopressin systems have also been tested on escalated alcohol drinking in the vapor model. The vasopressin V_{1b} receptor antagonist SSR149415 (Edwards et al., 2012) and cannabinoid receptor antagonist SR141716A (Rodríguez de Fonseca et al., 1999) both injected acutely reduced alcohol self-administration in dependent but not nondependent rats.

Additionally, alcohol vapor exposure reduces cell proliferation and survival in the mPFC (Richardson et al., 2009). Inhibition of matrix metalloproteinases, which degrade the extracellular matrix and modulate learning processes, reduced the development of escalation of alcohol-self-administration produced by vapor exposure but had no effect once escalated levels of alcohol self-administration were already reached (Smith et al., 2011). These findings suggest that vapor-induced escalation of alcohol self-administration is dependent on learning processes and neuroadaptation in brain areas related to cognition.

Several systems stand out as potential medication targets for alcohol dependence, including CRF₁, GRs, κ opioids, and those affected by gabapentin (GABA and glutamate). Indeed, the work summarized above and in Table 1 has been important in guiding clinical trials for alcohol dependence (clinicaltrials.com). There are two ongoing clinical trials testing CRF₁ antagonists (GSK561679 and Pexacerfont) led by NIAAA Clinical Director Marcus Heilig. Dr. Barbara Mason at The Scripps Research Institute is leading a clinical trial of Korlym (i.e., mifepristone, a GR antagonist). Dr. Suchitra Krishnan-Sarin from Yale is leading a

study examining whether naltrexone's ability to bind κ -opioid receptors is related to its effectiveness. Finally, there are several clinical trials involving baclofen, including one led by Dr. Lorenzo Leggio at NIH examining the link between anxiety and craving in alcoholics. This model of increased alcohol self-administration in rats following chronic vapor exposure has helped to uncover important biological factors that can be directly tested in the clinic.

Advantages and Limitations of this Model

There are several important advantages of this model of increased ethanol self-administration in dependent rats. For example, in the vapor model, the experimenter controls alcohol exposure to generate the desired range of blood alcohol levels (Gilpin et al., 2008a) to successfully produce alcohol dependence. Initially, alcohol vapor is set to low levels and then progressively increased over time. Different from other methods of dependence, including the liquid diet, this gradual increase in alcohol vapor allows the animal to develop tolerance to alcohol without having any noticeable detrimental health effects (e.g., weight loss or hypophagia). This model presents good reliability and predictive validity, arguably the only necessary and sufficient criteria for the evaluation of an animal model (Edwards & Koob, 2012; Geyer & Markou, 1995). As described above, the vapor model reliably leads to increases in alcohol intake and compulsive-like drinking and produces many other physical and motivational symptoms reminiscent of human alcohol dependence. This model has predictive validity in that it allows one to make predictions about the human condition generally and more specifically to identify drugs with potential therapeutic value. For example, drugs that are used to treat alcohol dependence in humans also reduce dependence symptoms in alcohol vapor-exposed rats. Probably most importantly, the publications listed in Table 1 are cited as rationale for ongoing clinical trials with new potential medications (as described above).

Exposure to alcohol vapor is imposed and, therefore, a criticism of this model has limited face validity given that humans usually do not breathe alcohol to become dependent nor are they forced to ingest alcohol. This disadvantage is partially balanced by the fact that rodents have a much shorter lifespan than humans, and therefore the length of exposure to high blood alcohol levels needs to be compressed in rodents. Unless genetically selected alcohol-preferring rats are used, getting sustained high blood alcohol levels would be difficult without experimenter influence.

The operant alcohol self-administration procedure also possesses advantages and limitations. Different from voluntary home-cage alcohol drinking, operant self-administration requires a relatively long training period, and not all animals will display stable levels of responding for alcohol. The investigator must establish an operant behavior (lever pressing) that most rats will not readily perform for a fluid that most rats will not readily consume. Therefore, the use of highly palatable sweet solutions or lengthy sessions is necessary to train rats to lever press and consume the delivered ethanol solution. However, once stable responding levels are achieved, operant self-administration is very versatile in terms of behavioral assessment. Less demanding schedules of reinforcement, such as FR1, are used to measure alcohol intake in a condition of low motivational requirement ("low price," "low

workload”). Progressive-ratio schedules of reinforcement measure the willingness of an animal to work to obtain alcohol (“high price,” “high workload”), which is used as an index of reward efficacy or compulsive-like behavior (Hodos, 1961). Continued alcohol use despite negative consequences is another important aspect of compulsive behavior in alcohol dependence that can be modeled with punishment schedules. For example, the alcohol solution can be adulterated with aversive substances (e.g., quinine is a bitter substance disliked by rodents; Wolffgramm & Heyne, 1995), or lever presses for alcohol can be paired with mild footshock to cause stress.

Another major advantage of this model is its utility in investigating self-administration following periods of protracted withdrawal. While most studies have investigated the effects of biological manipulations on alcohol self-administration during acute alcohol withdrawal, it is critical to investigate the effects of drugs during protracted alcohol withdrawal when the acute withdrawal symptoms have dissipated and relapse risk is highest. Escalated alcohol self-administration (Gilpin et al., 2008a b; Roberts et al., 2000; Sommer et al., 2008; Valdez et al., 2002; Vendruscolo et al., 2012) and neuroadaptations in reward-/stress-related brain regions (Francesconi et al., 2009; Vendruscolo et al., 2012) have been observed in rats with a history of alcohol vapor exposure after detoxification (3–8 weeks post removal from alcohol vapor). Unfortunately, as of yet, very few pharmacological studies have focused on protracted withdrawal. The NPY Y₂ receptor antagonist BIIE0246 reduced alcohol self-administration during protracted withdrawal in rats with a history of alcohol vapor exposure (Rimondini et al., 2005). Recently, we reported that chronic GR antagonism with mifepristone blocked escalated alcohol drinking (FR1) and compulsive-like responding (PR) during protracted withdrawal (Vendruscolo et al., 2012). It is not clear at this time how long lasting the craving and increased self-administration is in this model and how well this will model the human condition, but initial investigations have been promising.

Overall, alcohol vapor-exposed rats display increased intake of alcohol (FR1 test) and compulsive-like behavior toward alcohol (PR and adulteration tests) compared with nondependent rats, and this appears to be maintained following the acute withdrawal phase. Therefore, while there are a few areas of weakness, the combination of the vapor model with operant self-administration in rats constitutes an excellent tool for the study of the neurobiological basis of alcohol dependence.

Future Directions

The majority of studies examining alcohol self-administration have used nondependent animals. Effective targets and treatment development ideally should be investigated using dependence models, which have better predictive validity for alcoholism. The literature reviewed above describes several biological systems important in alcohol dependence and the way these have contributed to ongoing clinical trials. However, numerous issues remain to be addressed. These include genetics, gender, and age, as well as craving and long-term impacts on behavior.

Most studies described above used outbred Wistar rats, whose behavioral reactions could be considered adequate from the adaptive point of view because they may represent those of

the heterogeneous human population. However, the use of genetically defined inbred rat strains (e.g., Vendruscolo et al., 2006) and selectively bred rat lines may facilitate the identification of genetic mechanisms underlying susceptibility or resistance/resilience to alcohol dependence. Additionally, the use of rat strains displaying contrasting emotional and cognitive responses, such as high and low anxiety-like behavior, depressive-like behavior, impulsivity, behavior inhibition, and working memory, constitutes a useful tool to gain knowledge regarding behavioral characteristics that promote drinking and dependence.

While female rodents are beginning to make their way into alcohol studies (discussed in other reviews in this special issue), every one of the studies listed in Table 1 only employed males. Although alcohol dependence is still more prevalent in men, some evidence suggests that women initiate use earlier and progress faster to dependence and therefore represent a vulnerable population that has different and very understudied characteristics (Zilberman et al., 2003). For example, several studies showed that the female brain might be more sensitive to the degenerative effects associated with alcohol dependence (i.e., brain area volume reductions) and that women seek treatment earlier in their drinking history than males (reviewed by Sharrett-Field et al., 2013). Future studies using vapor-induced increases in operant ethanol self-administration need to include female rats.

The age of alcohol drinking onset (or alcohol exposure) is another critical issue to be studied. Few studies have investigated the consequences of adolescent alcohol exposure, which is a period of intense brain maturation and the time when humans start binge drinking, on subsequent alcohol-related maladaptive behavioral and pharmacological changes. Interestingly, Slawecki and Betancourt (2002) reported that exposure of adolescent rats (postnatal day 30–40) to intermittent alcohol vapor exposure did not predispose them to increased alcohol self-administration later in adulthood (> 3 months old). Moreover, the effect of a stressor on alcohol self-administration was not different in animals with a history of alcohol vapor exposure compared with control rats. Binge drinking of a sweetened alcohol solution during adolescence leads to increased alcohol self-administration in adulthood, regardless of alcohol vapor exposure in adulthood (Gilpin et al., 2012). Additionally, pharmacological and environmental manipulations (e.g., exposure to psychostimulant drugs or overconsumption of palatable solutions) during adolescence have been shown to produce changes in alcohol intake later on in adulthood (Vendruscolo et al., 2008, 2011) in nondependent rats. Whether similar effects would be observed in dependent rats remains to be investigated. Finally, the consequence of prenatal alcohol exposure on the vulnerability to alcohol dependence in adulthood is an area still needing further investigation.

Alcohol craving and its role in relapse is a critical area of study (Martin-Fardon & Weiss, 2013). There are several models used to investigate craving-like behavior in rodents, including conditioned reinstatement, stress-induced reinstatement, and conditioned place preference. These have begun to be combined with dependence models to better allow for the study of the chronic relapsing nature of addiction. For example, Liu and Weiss (2002), Ciccocioppo et al. (2003), and Kufahl et al. (2011) trained animals to associate olfactory discriminative stimuli with the availability of alcohol. Once stable responding was reached, the rats underwent extinction sessions without the discriminative stimuli, in which lever

presses were no longer reinforced with alcohol. This procedure produced a progressive reduction of operant responding. Upon presentation of the previously paired discriminative stimulus predictive of alcohol, the rats reinstated lever-press responding even in the absence of alcohol. The dopamine D₁ receptor antagonist SCH-23390 and dopamine D₂ receptor antagonist eticlopride dose-dependently blocked this effect. The same animals were exposed to alcohol vapor and tested again for reinstatement of alcohol-seeking behavior during protracted withdrawal. The induction of dependence did not alter the magnitude of reinstatement, but the effect of the antagonists was increased during protracted alcohol withdrawal (Liu and Weiss, 2002). However, Ciccocioppo et al. (2003) reported that the preferential μ -opioid receptor antagonist naltrexone reduced reinstatement in animals with a history of alcohol vapor exposure and control rats, but the effect was less pronounced in rats with a history of intermittent alcohol vapor exposure. Using similar procedures, Kufahl et al. (2011) reported that the mGluR2/3 agonist LY379268 dose-dependently reduced cue-induced reinstatement in nondependent rats and in rats with a history of alcohol dependence, but the effect was more pronounced in the latter group.

To date, the majority of studies have been conducted during acute/early alcohol withdrawal. In humans, the acute symptoms of alcohol withdrawal (e.g., anxiety, agitation, shaking, tremor, headache, sweating, nausea, confusion, hallucinations, delirium tremens, seizures, high blood pressure, and fever) are often managed with benzodiazepines or barbiturates until the symptoms have dissipated (Stehman & Mycyk, 2013). However, a major challenge in the alcohol field is to control the long-lasting symptoms of alcohol withdrawal (e.g., alcohol craving, increased anxiety and depressive symptoms, increased stress reactivity, hypohedonia, disorientation, nausea, headache, and insomnia), which lead to high rates of relapse in alcoholics after detoxification. Studies with models of alcohol dependence and withdrawal and operant self-administration have just started to uncover the molecular and pharmacological mechanisms underlying protracted alcohol withdrawal.

Here, we described results obtained from studies of rats trained to lever press for alcohol and made dependent, mostly through vapor exposure. These studies have already provided critical information regarding alcohol dependence and are helping to improve our knowledge in the field, which will ultimately contribute to the development of better strategies of prevention, diagnosis, and treatment of alcoholism.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

(A) Operant responding for alcohol across the second 12-h test period by dependent (alcohol vapor) and nondependent (Air) rats. (B) Blood alcohol levels taken during this second test (left) as well as at the same time points in a third test in which rats were not able to lever-press for ethanol (right). (C) Alcohol withdrawal severity obtained during test 2 (while rats were allowed access to alcohol in the operant boxes) and test 3 (while in home cages). Taken from Roberts et al. (1996), with permission.

Fig. 2.

Specific increase in alcohol intake and compulsive-like drinking in alcohol vapor-exposed rats during acute alcohol withdrawal. (A) Alcohol intake in g/kg of body weight before (pre-vapor) and during alcohol vapor exposure on an FR1 schedule of reinforcement. (B) Breakpoint for alcohol achieved in a progressive-ratio test. (C) Persistence of alcohol drinking despite the aversive bitter taste of quinine (added to the alcohol solution). (D) Intake (in mL/kg of body weight) of a saccharin (0.004%, w/v) solution (FR1). * $p < 0.05$, significant difference between dependent and nondependent; # $p < 0.05$, significant difference from 0. Modified from Vendruscolo et al. (2012), with permission.

Table 1

Drug effects on operant alcohol self-administration and reinstatement of alcohol seeking in rats exposed to alcohol vapor (Dependent) and control (Nondependent) rats.

Author(s)	Year	Compound/action	Nondependent	Dependent	Withdrawal time
Liu and Weiss	2002	SCH23390 (D ₁ antagonist)	↓ (R)	↓(R)	21 days
Liu and Weiss	2002	Eticlopride (D ₂ antagonist)	↓ (R)	↓(R)	21 days
Ciccocioppo et al.	2003	Naltrexone (μ opioid antagonist)	↓ (R)	↓(R)	1 week
Kufahl et al.	2011	LY379268 (mGluR2/3 agonist)	↓ (R)	↓(R)	1 week
Richardson et al.	2008	MPZP (CRF ₁ antagonist)	–	↓	6–8 h
Valdez et al.	2002	D-Phe-CRF _{12–41} ICV (CRF antagonist)	–	↓	2 h
Funk et al.	2006	D-Phe-CRF _{12–41} (CRF antagonist) in CeA	–	↓	2 h
Funk et al.	2006	D-Phe-CRF _{12–41} (CRF antagonist) in NAc or BNST	–	–	2 h
Roberto et al.	2010	R121919 (CRF antagonist)	↓	↓	6–8 h
Funk and Koob	2007	Urocortin 3 (CRF ₂ agonist)	–	↓	2 h
Vendruscolo et al.	2012	Mifepristone (GR antagonist)	–	↓	6–8 h
Vendruscolo et al.	2012	Mifepristone (GR antagonist)	–	↓	3–6 weeks
Thorsell et al.	2005	Neuropeptide Y (ICV)	NT	↓	2 weeks
Gilpin et al.	2011	NPY (ICV)	↓	↓	6 h
Kallupi et al.	2013	JNJ-31020028 (systemic) and BIIIE0246 (intra- CeA): NPY Y ₂ antagonists	–	–	6–8 h
Roberts et al.	1996	Muscimol (GABA _A agonist)	–	↓	0–12 h
Roberto et al.	2008	Gabapentin	–	↓	2 h
Walker and Koob	2007	Baclofen (GABA _B agonist)	↓	↓	6 h
Walker et al.	2011	nor-binaltorphimine (κ opioid antagonist)	–	↓	6 h
Kissler et al.	2013	nor-BNI (κ opioid antagonist) in the CeA	–	↓	6–10 h
Kissler et al.	2013	CTOP+Naltrindole (μ and δ opioid antagonist) in the CeA	↓	–	6–10 h
Kissler et al.	2013	Nalmefene (μ and κ opioid antagonist) in the CeA	↓	↓	6–10 h
Nealey et al.	2011	Nalmefene (μ and κ opioid antagonist) and CTOP+Naltrindole (μ and δ antagonist) in the NAc shell	↓	↓	6 h
Nealey et al.	2011	nor-binaltorphimine (κ opioid antagonist) in the NAc shell	–	↓	6 h
Walker and Koob	2008	nor-binaltorphimine (κ opioid antagonist) (ICV)	–	↓	6 h

Author(s)	Year	Compound/action	Nondependent	Dependent	Withdrawal time
Walker and Koob	2008	Nalmefene (μ and κ opioid antagonist) and naltrexone (μ opioid antagonist)	↓	↓	6 h
Sabino et al.	2009	BD-1063 (σ antagonist)	–	↓	6 h
Gilpin and Koob	2010	Propranolol (β antagonist)	↓	↓	6 h
Walker et al.	2008	Prazocin (α_1 antagonist)	↓	↓	6 h
Edwards et al.	2012	SSR 149415 (vasopressin V_{1b} antagonist)	–	↓	6 h
Rodríguez de Fonseca et al.	1999	SR141716A (CB_1 antagonist)	–	↓	6–8 h
Smith et al.	2011	FN-439 (matrix metalloproteinases inhibitor: ICV)	NT	↓	6 h

NT, not tested; R, cue-induced reinstatement.