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Rodent models and mechanisms of voluntary binge-like ethanol consumption: Examples, opportunities, and strategies for preclinical research

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Abstract

Binge ethanol consumption has widespread negative consequences for global public health. Rodent models offer exceptional power to explore the neurobiology underlying and affected by binge-like drinking as well as target potential prevention, intervention, and treatment strategies. An important characteristic of these models is their ability to consistently produce pharmacologically-relevant blood ethanol concentration. This review examines the current available rodent models of voluntary, pre-dependent binge-like ethanol consumption and their utility in various research strategies. Studies have demonstrated that a diverse array of neurotransmitters regulate binge-like drinking, resembling some findings from other drinking models. Furthermore, repeated binge-like drinking recruits neuroadaptive mechanisms in mesolimbocortical reward circuitry. New opportunities that these models offer in the current context of mechanistic research are also discussed.

Keywords

Introduction

With a financial burden estimated in excess of $223 billion in the United States alone (Bouchery et al., 2011), ethanol abuse has widespread negative consequences for public health and has been implicated in 79,000 deaths annually (Stahre et al., 2004). Not all excessive ethanol consumption is the same, however, and different forms of aberrant alcohol use are associated with different drinking trajectories and negative consequences (Cleveland et al., 2013; Gueorguieva et al., 2012; King et al., 2011; Mota et al., 2013). It is therefore important to explore the major subtypes of problematic alcohol use and their associated mechanisms and consequences.

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One such subtype, binge drinking, is a hazardous, yet common occurrence in the United States. The National Institute on Alcohol Abuse and Alcoholism defines binge drinking (BD) as a pattern of drinking that brings blood ethanol concentration (BEC) levels to 80 mg/dL in a short period of ~2 hours which can typically be achieved after 4 drinks for women and 5 drinks for men. This level of ethanol consumption lies in between light or social consumption and the extreme levels typically seen in dependent individuals. BD is also defined by periodic, rather than continuous drinking and has been associated with increased risk of car accidents, sexual assault, personal injury, and ethanol poisoning. Moreover, heavy or frequent BD may lead to a loss of control over alcohol consumption, and the development of alcohol use disorders (Courtney and Polich, 2009). Whether or not BD is associated with a progression towards *dependence* in the clinical literature is not yet clear (Chassin et al., 2002; Courtney and Polich, 2009; Hasin and Beseler, 2009; King et al., 2011), however the new spectrum of alcohol use disorder diagnosis presented in the DSM-5 is arguably more inclusive for BD behavior than was previously seen with the DSM-IV TR. Nevertheless, one in six adults in the United States reported engaging in BD about 4 times per month in 2010, consuming roughly 8 drinks in each binge episode (CDC, 2012). Thus, there is a critical need to better understand the neurocircuitry engaged by BD, as well as how this neurocircuitry is altered by repeated bouts of BD, for informed progress in the treatment, intervention, and prevention of alcohol use disorders.

Although clinical research has provided critical information regarding the risks and consequences of BD, human studies cannot meticulously examine the biological and chemical underpinnings of BD due to ethical limitations. Rodent models have therefore been extremely valuable in efforts to understand the neurobehavioral mechanisms and consequences of binge ethanol consumption. In this review, we will first describe the available binge-like drinking rodent models and discuss their application in mechanistic research, highlighting findings in key neurotransmitter systems. Finally, we will offer suggestions for future utility of these models and how they can continue to advance our understanding of the neurobehavioral and genetic mechanisms underlying binge ethanol consumption.

Rodent models of voluntary binge-like ethanol consumption

The predominant method of assessing ethanol drinking in rodents has been a two-bottle choice paradigm wherein the animal concurrently has access to an ethanol-containing solution and water. Often employed as a continuous-access model, this design allows the researcher to determine preference for the ethanol solution over water as well as total fluid intake in the animal's home cage under normal conditions (save for isolated housing). Certain mouse and rat genotypes will consume appreciable amounts of ethanol in this paradigm (Eriksson, 1970; McClearn and Rodgers, 1959; Wahlsten et al., 2006), however, the continuous nature of this drinking paradigm makes it difficult for the researcher to determine when peak drinking/BEC occurs. Indeed, a major limitation is that even animals demonstrating a significant preference for ethanol over water rarely achieve pharmacologically-relevant BECs in this paradigm (80 mg/dl) (Dole and Gentry, 1984; Linseman, 1987).

Limiting ethanol access to a discrete time period (typically 1-4 hours each day) has been found to produce high ethanol intakes in rodents and BECs ≥ 80 mg/dl in as little as 30 minutes and produce measurable behavioral intoxication (Bell et al., 2006b; Crabbe et al., 2011; Cronise et al., 2005; Rhodes et al., 2005). As these observations reflect the NIAAA definition of binge drinking, a number of these models have been referred to as 'binge-like drinking models.' Studies in mouse behavioral and quantitative genetics also suggest that this binge-like drinking phenotype is not completely analogous to the continuous, 2-bottle choice drinking phenotype (Crabbe et al., 2011; Fritz et al., 2014b; Iancu et al., 2013). This is an important point to consider as different genetic factors may predispose individuals for binge ethanol drinking, specifically.

A wide variety of approaches to modeling voluntary BD in rodents exist, including voluntary home cage drinking, operant paradigms, and dependence-induced drinking. In the current review, the authors have elected to focus on pre-dependent, voluntary home cage consumption paradigms in mice for a number of reasons. First, operant paradigms necessitate an appetitive response component, making interpretation of effects on binge-like ethanol *consumption*, specifically, difficult to ascertain. Second, dependence-induced models of BD require animals to be repeatedly exposed to stressful ethanol vapor inhalation for up to 12-16 hours per day. The authors argue that modeling BD in this manner lacks face and construct validity as this chronic vapor exposure, not prior voluntary ethanol consumption, is likely responsible for producing the BD phenotype and therefore reflects the maintenance of already established dependence. In addition, a protracted history of binge-like ethanol consumption in a mouse model of BD did not produce well-established behavioral markers of ethanol dependence in rodents (Cox et al., 2013), although an elevation of ethanol intake was observed. With these considerations, the scope of this review is to examine BD as a pre-dependent mechanism of problematic ethanol consumption, itself. In addition, the possibility of whether prolonged BD may usher in a *transition* to dependence is discussed.

Drinking-in-the-Dark

Drinking-in-the-Dark (DID) was developed using the highest ethanol-drinking inbred mouse strain, C57BL/6J (B6). This drinking paradigm takes advantage of the most active circadian period in mice (3 hrs into the dark cycle) by replacing the animal's water bottle with an unsweetened, 20% (v/v) ethanol solution for a short period of 2-4 hrs each day. B6 mice will typically consume ~4-6 g/kg of ethanol by the 2nd DID session (i.e. 2 successive days), with a significant proportion reaching BECs in excess of 100 mg/dl with repeated exposures (Fritz et al., 2014a; Lyons et al., 2008; Rhodes et al., 2005). DID has been validated as a binge-like drinking model as mice reach these intoxicating BECs in a short period of time (2-4 hrs) and display behavioral markers of intoxication (Fritz et al., 2014a; Linsenbardt et al., 2011; Rhodes et al., 2007). Furthermore, B6 mice have been demonstrated to develop functional and metabolic tolerance (Fritz et al., 2014a; Linsenbardt et al., 2011) as well as a greater propensity for locomotor sensitization to ethanol (Linsenbardt et al., 2011; Tarragón et al., 2012) following repeated cycles of DID, perhaps modeling valid markers of protracted ethanol abuse. Concerning whether DID produces ethanol dependence, a previous study demonstrated that although repeated DID cycles increased later ethanol intake (Cox et al.,

2013), this prolonged consumption did not produce other dependence-like phenotypes typically demonstrated by rodents following ethanol vapor withdrawal (i.e. anxiety-like behavior, convulsions, ataxia). The authors concluded that DID is therefore a pre-dependent assessment of binge-like ethanol consumption although the elevation of intake with prolonged exposure may suggest a transition towards dependence. Future studies are encouraged to address this question by further extending the ethanol exposure period. Finally, genotype is an important mediator of propensity to engage in DID (Rhodes et al., 2007), and consistent with anecdotal evidence from the human literature, adolescent B6 mice consume greater amounts of binge-like ethanol than adults using DID procedures (Moore et al., 2010).

There are numerous variations of DID, particularly relating to the schedule of presentation. The original version (Rhodes et al., 2005) offers 2-hr ethanol access for 3 days, and a 4-hr access period on day 4. This longer access period effectively allows for greater overall ethanol intake as mice will roughly double their consumption to doses of ∼8 g/kg. Others have adapted the schedule to draw out to ∼14 days or longer, however only 2-hr access is used. The interested reader is referred to Thiele et al. (2014) for details on setting up and using DID.

DID-Multi-Scheduled Access

DID multi-scheduled-access (DID-MSA) offers 3-4 daily periods of 1-hr limited access to two concentrations of ethanol concurrently (15 and 30% v/v; tap water is also freely available), spaced 2-3 hrs apart. Daily ethanol intake equivalent to what is reached if the ethanol access is continuous has been observed in selectively-bred high alcohol drinking P rats (Bell et al., 2006a; Bell et al., 2011) with mean BECs as high as 120 mg/dl after the first hour of access (Bell et al., 2006b). It should be noted that continuous 2-bottle choice access to 10% ethanol and water was given before the initiation the DID-MSA protocol. Findings from gene expression studies suggest that binge-like drinking in this paradigm produces significant alterations in protein expression related to cellular structure and function in the nucleus accumbens and amygdala (Bell et al., 2006a; McBride et al., 2010). Moreover, P rats drinking ethanol in a continuous access paradigm exhibited substantially more protein alterations in the amygdala whereas DID-MSA produced more pronounced alterations in the nucleus accumbens (Bell et al., 2006a). These findings suggest that this binge-like drinking phenotype produced unique, regionally-specific changes in protein expression.

Our lab has also explored the utility of DID-MSA in B6 mice. Using a variation of the paradigm, mice were given 3 daily 1-hr access periods, separated by 2 hrs, to a single bottle of 20% (v/v) ethanol for 14 days. Towards the end of the experiment, daily ethanol intakes were 8 g/kg and BECs were 80 mg/dl following the final hour of access (Melón et al., 2013). Mice that consumed ethanol in this experiment also exhibited significant ataxia on the balance beam apparatus. We have also observed similar binge-like ethanol intake in adolescent male and female selectively bred high alcohol-preferring (HAP) mice from the first replicate of selection (HAP1) using this paradigm (unpublished observations). Thus, DID-MSA also has potential as a mouse model of binge ethanol drinking, although further

study regarding the appropriateness of the ethanol concentration offered and the duration of the rest periods is needed.

Limited access, 2-bottle choice

One of the limitations of the standard DID procedure is that animals only have access to an ethanol solution during the test period. This may be an issue for researchers interested in ethanol preference or total fluid intake. However, the 2-bottle choice paradigm also appears to be sensitive to limited-access procedures. If offered a choice between 10% or 20% (v/v) ethanol and water, B6 mice have been shown to consume ~2-6.5 g/kg of ethanol and reach BECs ~50-90 mg/dl in a 2-4 hr period as well as demonstrate an ethanol preference of ~60-80% (Cozzoli et al., 2014c; Ramaker et al., 2014; Rhodes et al., 2007). Thus, although concurrent access to water does appear to reduce ethanol intake in a limited access paradigm to some degree relative to the standard DID paradigm described above, BECs at the 'binge' level (80 mg/dl) can still be observed.

Scheduled High Alcohol Consumption

The scheduled high alcohol consumption (SHAC) paradigm, also developed using the B6 strain, models binge consumption (~2 g/kg) in a very short period of time (~30 min) and has been shown to produce intoxicating BECs 100 mg/dl (Szumlinski et al., 2007a; Tanchuck et al., 2011). SHAC produces high ethanol intake by conditioning mice to consume fluids on a schedule. The basic procedure restricts fluid access to 4-10 hrs per day with only a portion of this period (~30 min) offering ethanol access. Ethanol is only offered during this designated fluid access period every 3 days with each ethanol access period constituting one SHAC 'cycle'. The procedure also utilizes a lower concentration of ethanol than typically used in DID (5% v/v versus 10-20% v/v). Similar to DID, female B6 mice exhibit behavioral intoxication following ethanol access (Cronise et al., 2005). Although B6 mice are the most impressive drinkers in this procedure, genetically heterogeneous mice (ethanol-induced Withdrawal Seizure-Control mice; WSC) have been shown to reach BECs 100 mg/ml following SHAC access (Finn et al., 2005), demonstrating that this model can produce binge-like drinking in mouse genotypes other than B6.

Selected Rodent Lines

High Drinking in the Dark (HDID) mice have been selectively bred from the heterogeneous HS/Npt mouse stock for high BECs following 4-hr ethanol access in a 2d DID procedure. These mice will consume ethanol in a binge-like manner, engaging in large drinking bouts (Barkley-Levenson and Crabbe, 2012) and can reach BECs in excess of 120 mg/dl in a 4-hr DID session (Crabbe et al., 2014). Rat lines have also been selected for divergent ethanol intake in a limited access paradigm. The high and low drinking lines, referred to as HARF and LARF (High/Low Alcohol Research Foundation) (Lê et al., 2001), consumed on average 1.2 g/kg and 0.6 g/kg of ethanol, respectively in 30 minute sessions. Unfortunately, these rat lines are now extinct and no genetic rat model bred specifically for binge-like ethanol drinking currently exists.

For researchers specifically interested in genetic predisposition for BD, HDID mice currently offer the most appropriate model. However it is worth noting that HAP1 mice have

also been observed to demonstrate impressive ethanol consumption via DID, reaching BECs ~100 mg/dl, suggesting at least some genetic overlap between high 2-bottle choice and binge-like ethanol drinking (Crabbe et al., 2011). Thus, both HDID and HAP mice offer powerful tools for researchers interested in phenotypes associated with a genetic propensity for binge-like drinking.

Mechanisms of binge-like alcohol consumption

Dopamine

The most thoroughly studied neurotransmitter in drug/ethanol abuse research, dopamine (DA), contributes to the reinforcing and rewarding effects of ethanol (Di Chiara, 1999; Koob, 1992; Tupala and Tiihonen, 2004). Although, it is important to highlight that apart from psychostimulants, more recent research suggests that DA may play a more diminished role in drug and ethanol reinforcement than previously thought (Pierce and Kumaresan, 2006). A well-known hypothesis for ethanol and drug abuse is an underlying hypofunction of the dopamine system (Blum et al., 2015), with consumption of these substances theoretically reflecting an attempt to normalize or elevate DA signaling. Ethanol consumption has been demonstrated to enhance DA release in rodent (Doyon et al., 2003; Melendez et al., 2002; Middaugh et al., 2003) and human (Boileau et al., 2003), nucleus accumbens, a mesolimbic area with known involvement in substance use/abuse phenotypes. This DA response is thought to reflect disinhibition of DA neurons in the VTA by ethanol, resulting in an increased DA efflux from neurons projecting to the accumbens (Froehlich and Wand, 1996). Systemic administration of DA agonists has been shown to decrease ethanol intake in rodents across a variety of genotypes and paradigms (Cohen et al., 1999; Dyr et al., 1993; Ng and George, 1994; Silvestre et al., 1996). Furthermore, a link between dopamine D2 receptor levels/function and ethanol abuse/dependence has been demonstrated in the clinical literature (Volkow et al., 2015; Volkow et al., 1996) and in rodent models (Bice et al., 2008; McBride et al., 1993; Ng et al., 1994; Phillips et al., 1998; Thanos et al., 2005; Thanos et al., 2001); however, whether these alterations reflect predisposing phenotypes or neuroadaptation as a consequence of chronic ethanol exposure is unclear.

Binge-like drinking also appears to have a DA component in rodents, although it has been surprisingly understudied. An elevation of extracellular DA levels in the nucleus accumbens of male B6 mice during SHAC ethanol drinking was observed using *in vivo* microdialysis procedures (Szumlinski et al., 2007a). Pharmacologic inhibition of the DA transporter (DAT) via systemic administration of the drug GBR 12909, theoretically increasing extracellular DA levels, significantly reduced ethanol intake via DID in male B6 mice (Kamdar et al., 2007). However, this effect was not ethanol-specific as GBR 12909 also reduced sugar water intake, suggesting that global inhibition of the DAT may simply influence hedonic processes. Interestingly, GBR 12909 infused directly into the accumbens shell of P rats did not influence ethanol intake in a procedure resembling 2-bottle DID (Engleman et al., 2000). In an attempt to clarify discrepancies in rodent studies about the directionality of the relationship between D2 receptor levels and ethanol consumption, Bulwa et al. explored the contribution of the two different D2 receptor isoforms: the long DA D2 receptor isoform (D2LR) and the short (D2SR) isoform. Mice lacking (knockout;

KO) D2LR, and consequently having more D2SR, consume significantly more ethanol via the classic DID procedure (Bulwa et al., 2011). Although the roles of the isoforms in ethanol sensitivity is not yet known, functional and behavioral differences associated with these receptor subtypes (Bulwa et al., 2011; Fetsko et al., 2003; Smith et al., 2002; Usiello et al., 2000; Wang et al., 2000) highlight their potential to differentially influence sensitivity to ethanol and ultimately, binge-like drinking.

Acute systemic injections of ethanol have been consistently demonstrated to produce DA efflux in the nucleus accumbens of rats and mice (Imperato and Di Chiara, 1986; Szumlinski et al., 2007a; Yim and Gonzales, 2000; Yim et al., 2000), mirroring what was seen during binge-like drinking in SHAC (Szumlinski et al., 2007a). Furthermore, the DA response to this acute injection was not influenced by the extent of a binge-like ethanol consumption history (Szumlinski et al., 2007a). These findings demonstrate that ethanol exposure does not need to be contingent with consumption to elevate extracellular DA, suggesting this observation is simply a reflection of ethanol's pharmacological effects. Although GBR 12909 itself has not been extensively studied, activation of DA receptors via agonists has been shown to reduce ethanol intake across a variety of rodent genotypes and paradigms (Cohen et al., 1999; Dyr et al., 1993; Ng and George, 1994; Silvestre et al., 1996). However, sucrose or saccharin solution intake was also observed to be reduced in a number of these studies (Cohen et al., 1999; Dyr et al., 1993; Kamdar et al., 2007). One interpretation is that this elevated DA signal may serve as a 'substitute' for the DA response sought by drugs/ ethanol or natural rewards and this interpretation is strengthened by the observation that DA antagonists can decrease ethanol intake (Dyr et al., 1993; Ng and George, 1994). In other words, DA agonists may "replace" the augmented DA signal produced by ethanol or other natural rewards, and antagonists may prevent the occurrence of this augmented signal, thus offering a possible explanation of why both DA receptor antagonists and agonists have been observed to reduce the intake of ethanol and other natural reinforcers. Considering how much is known about DA in other alcohol abuse rodent models (Tupala and Tiihonen, 2004), far more work needs to be conducted with these binge drinking models to determine the role of DA signaling in binge ethanol consumption.

Glutamate

Ethanol has been shown to influence pre- and postsynaptic glutamate signaling in the central nervous system (Chandler, 2003). An acute exposure to ethanol enhances extracellular glutamate levels in the nucleus accumbens (Lominac et al., 2006; Moghaddam and Bolinao, 1994) and VTA (Xiao et al., 2008), and repeated injections have been shown to increase basal glutamate levels (Melendez et al., 2005) as well as sensitize the glutamate response to ethanol in the accumbens (Szumlinski et al., 2005) and the central amygdala (Roberto et al., 2004). A well-known pharmacological effect of ethanol is inhibition of the N-methyl-D-aspartate (NMDA) receptor (Hoffman et al., 1989) and this system is implicated in neuroplasticity associated with ethanol abuse/depenence phenotypes (Chandler, 2003; Chandler et al., 1998). Indeed, a body of evidence suggests that protracted ethanol exposure produces a compensatory increase in glutamate levels and excitability, effects implicated in withdrawal-induced seizures and ethanol-seeking (Bäckström et al., 2004; Chandler et al., 2006).

Binge-like ethanol intake has been demonstrated to significantly increase extracellular glutamate levels in the nucleus accumbens of male B6 mice during ethanol consumption in a SHAC procedure, although this was only observed in mice that had previously consumed ethanol over repeated SHAC cycles (Szumlinski et al., 2007a). Therefore, this enhanced glutamate response appears to reflect ethanol experience-dependent neuroadaptation. Interestingly, the antiepileptic drug levetiracetam, which potently inhibits vesicular release of glutamate (Meehan et al., 2011; Meehan et al., 2012), has been shown to *increase* ethanol intake in male B6 mice during a 4-hr DID procedure (Fish et al., 2014). In conjunction with the microdialysis data described above, this observation may reflect a compensatory increase in ethanol intake to oppose the drug-induced reduction in glutamatergic tone. Binge-like ethanol intake has also been demonstrated to alter the number and makeup of glutamate receptors. Six cycles of SHAC elevated NR2a and NR2b NMDA receptor subunit levels in the accumbens of male B6 mice (Cozzoli et al., 2009). A subsequent experiment found a 30 day history of 2-hr DID elevated NR2B and mGluR5 levels in the accumbens shell of male B6 mice. Increased expression of mGluR1 was also observed in the accumbens shell of ethanol-naive high drinking HDID-1 mice relative to their progenitor stock HS/Npt counterparts (Cozzoli et al., 2012), and these findings were recently extended to the central nucleus of the amygdala using this 30 day DID approach (Cozzoli et al., 2014a).

Pharmacological studies have begun to elucidate the role of specific glutamate receptors in binge-like drinking. One of a handful of drugs currently approved to treat ethanol use disorders, acamprosate acts partly by blocking NMDA/metabotropic glutamate receptor (mGluR) signaling, and systemic administration significantly reduced DID ethanol intake in male B6 mice (Gupta et al., 2008). Systemic administration of mGluR5 antagonists reduces binge-like ethanol intake across multiple paradigms and mouse genotypes (Gupta et al., 2008; Tanchuck et al., 2011), however, a recent investigation suggests that their efficacy may be influenced by age and sex (Cozzoli et al., 2014b). Local infusion of an mGluR5 antagonist directly into the nucleus accumbens shell has also been shown to mirror this effect (Cozzoli et al., 2009). Although mGluR1 antagonism has not emerged a promising pharmacotherapy for alcohol consumption, the negative allosteric modulator JNJ-16259685 was recently shown to reduce the ethanol intake of male B6 mice in DID when infused into the accumbens shell (Lum et al., 2014). Furthermore, this drug effect was dependent on the scaffolding protein, Homer2, suggesting that mGluR1 and Homer2 interaction is necessary for the drug effect. A number of research groups have thoroughly explored the involvement of these complex interactions between glutamate receptors and their scaffolding proteins in binge-like drinking in mice. The interested reader is referred directly to a number of these studies as these interactions are beyond the scope of this review (Cozzoli et al., 2012; Cozzoli et al., 2009; Szumlinski et al., 2007b).

The enhanced accumbal glutamate response following repeated cycles of Binge-like drinking resembles what has been shown with chronic injection regimens, although no acute response to consumption was demonstrated. The observation that repeated SHAC consumption may usher in an enhanced glutamate response component associated with dependence could indicate repeated binge-like drinking may represent some facets of dependence transition, although this possibility must be more thoroughly explored. For

example, extending DID ethanol exposure to 10 cycles (40 total ethanol drinking sessions) produced no evidence of well-known dependence phenotypes (e.g. handling-induced convulsions, anxiety, ataxia) during withdrawal (Cox et al., 2013), although elevated ethanol intake was observed. The efficacy of type 1 glutamate receptor (mGluR1 and mGluR5) antagonism/inhibition in reducing binge-like drinking aligns with findings via operant paradigms (Bäckström et al., 2004; Hodge et al., 2006b; Schroeder et al., 2005) and knockout models (Bird et al., 2008) and has also shown regional specificity in the accumbens shell (Besheer et al., 2010). Collectively, these findings suggest that glutamatergic mechanisms regulate binge levels of ethanol intake in pre-dependent animals and protracted consumption can induce neuroplasticity associated with elevated glutamate signaling and alterations in receptor composition/number in key mesolimbic regions.

γ-Aminobutyric Acid (GABA)

Ethanol has been shown to act as a positive modulator of GABA signaling (Ariwodola and Weiner, 2004; Mihic et al., 1997; Olsen et al., 2007) and *in vitro* techniques have demonstrated that this effect can be both pre- and postsynaptic (Roberto et al., 2003). Ethanol facilitates GABA release and pharmacologically interacts with both $GABA_A$ and $GABA_B$ receptors (Kelm et al., 2011; Paul, 2006) and a variety of agonists/antagonists influence ethanol intake in rodents across numerous genotypes and paradigms (Kasten and Boehm, 2015; Liang et al., 2006; Moore et al., 2007; Smith et al., 1992; Tanchuck et al., 2011). Drug discrimination studies in rodents suggest a $GABA_A$ receptor component in the subjective pharmacological effects of ethanol at doses as low as 0.5-1.0 g/kg (Engel et al., 2001; Hodge et al., 2006a), doses that would be expected to produce translationally-relevant BECs. One theory for GABA's role in ethanol use is that ethanol-induced alterations in GABA signaling may serve as regulatory processes within the VTA, ultimately influencing the activity of DA neurons projecting to other mesolimbic regions such as the accumbens. Indeed, $GABA_A$ receptor antagonism within the VTA increases accumbal dopamine levels (Kalivas et al., 1990) and slows the acquisition of ethanol self-administration (Nowak et al., 1998). In addition, a single systemic injection of ethanol has been shown to predominately potentiate $GABA_A$ receptor transmission in DA neurons within the VTA (Melis et al., 2002). Interestingly, neuroplastic alterations in VTA DA neurons induced by administration of a $GABA_A$ receptor agonist were not associated with rewarding or reinforcing effects of the drug when either systemically or directly administered into the VTA of mice and non-human primates (Vashchinkina et al., 2012). This suggests that $GABA_A$ receptor transmission within the VTA is indeed involved in ethanol consumption and ethanol-induced neuroplasticity, although its association with ethanol self-administration is complex as it is not independently responsible for ethanol's rewarding/reinforcing effects.

Binge-like drinking in mice has also been shown to alter the responsivity of GABA signaling as a consequence of voluntarily consumed ethanol. The Szumlinski et al. microdialysis study (2007a) found that although the initial voluntary ethanol exposure for male B6 mice produced an increase in extracellular GABA in the accumbens, a repeated consumption history was associated with no such effect. Another group recently observed that a history of binge-like drinking via DID reduced the baseline frequency of $GABA_A$-receptor mediated inhibitory postsynaptic currents in medium spiny neurons located in the

dorsal striatum, evidence of a reduction in presynaptic GABA release (Wilcox et al., 2014). Furthermore, acute ethanol-induced GABA responses in these medium spiny neurons in water-exposed mice were not observed in mice with a DID history. These observations suggest that repeated voluntary binge-like drinking produces reductions in the GABA response to ethanol, perhaps indicating a mechanism of neurochemical tolerance.

Ethanol intake in binge-like drinking models is influenced by both ionotropic $GABA_A$ and metabotropic $GABA_B$ receptors. Systemic administration of $GABA_A$ receptor agonists has been repeatedly shown to decrease ethanol intake and associated BECs in DID paradigms (Melón and Boehm II, 2011; Moore et al., 2007; Ramaker et al., 2011; Ramaker et al., 2012). Local infusion of Ro 15-4513 (a $GABA_A$ receptor inverse agonist) into the posterior, but not anterior VTA, produced an ethanol-specific decrease in consumption in DID and associated BECs (Melón and Boehm II, 2011). This is noteworthy as these anatomical subdivisions of the VTA differ in their efferent projections to regions of mesolimbocortical circuitry. For example, most DA neuron projections to the medial prefrontal cortex and ventromedial striatum arise from the posterior VTA, whereas the anterior VTA prominently sends projections to the dorsal striatum and more lateral regions of the nucleus accumbens (Ikemoto, 2007; Oades and Halliday, 1987). With respect to ethanol's pharmacological effects, ethanol-preferring P rats will self-administer ethanol directly into the posterior, but not anterior VTA (Rodd-Henricks et al., 2000). This is particularly interesting in regard to the aforementioned hypothesis that a pharmacological component of ethanol reward/ reinforcement may be GABAergic disinhibition of VTA DA neurons. A prominent projection target of the VTA, the nucleus accumbens shell also regulates 2-bottle DID ethanol intake though $GABA_A$ processes as local infusion of the $GABA_A$ receptor partial agonist THIP significantly reduced ethanol consumption (Ramaker et al., 2014). Finally, THIP infused into the infralimbic cortex, a region associated with behavioral flexibility and fear extinction (Morgan and LeDoux, 1995; Ragozzino et al., 1999), has been shown to promote ethanol intake in DID and associated BECs (Fritz and Boehm II, 2014).

In a version of the SHAC procedure, the $GABA_B$ receptor agonist baclofen dose-dependently reduced ethanol intake in genetically heterogeneous male WSC mice (Tanchuck et al., 2011), and our group observed an inverted-U-shaped effect of systemic baclofen on ethanol consumption in a 1-hr DID procedure in male B6 mice (Moore et al., 2007). Recently, we have also shown that that the two different enantiomers of baclofen (S(-)- and R(+)) can bidirectionally alter DID ethanol intake with the far more potent R(+) enantiomer decreasing ethanol intake, and the less potent S(-)-enantiomer increases intake (Kasten and Boehm, 2015). It is unclear whether many previous systemic baclofen studies involved the racemate, or one of the individual R(+)-or S(-)- enantiomers as these distinctions were typically not made in the literature. In light of these findings, these enantioselective effects should be considered. The $GABA_B$ receptor positive allosteric modulator GS39783 was also found to significantly reduce ethanol intake in the first 15 minutes (where male B6 mice frontloaded over 40% of their total ethanol intake) of 2-hr DID (Linsenbardt and Boehm, 2014). Finally, local infusion of baclofen into the anterior, but not posterior VTA was also found to reduce ethanol intake in DID (Moore and Boehm II, 2009), and the aforementioned enantioselective effects of baclofen on DID ethanol

intake, with R(+) decreasing and S(-)- enhancing intake, were extended to the accumbens shell (Kasten and Boehm II, 2014).

Collectively, these findings suggest that acute binge ethanol intake/exposure can induce an acute increase in GABA signaling in the accumbens shell and striatum, however repeated voluntary exposure blunts these responses, perhaps indicating forms of neurochemical tolerance. Furthermore, pharmacological manipulation of both $GABA_A$ and $GABA_B$ receptors significantly influences binge-like drinking as seen with other drinking paradigms, although the direction of this effect appears dependent on the influence of particular mesocorticolimbic regions, and in the case of baclofen, on the specific enantiomer studied. Given ethanol's demonstrated role as a positive modulator of GABA receptor signaling, the extent to which GABA receptor manipulation in these key brain regions 'substitutes' for or interferes with the pharmacological effects of Binge-like ethanol intake should be further explored.

Nicotinic Acetylcholine System

The strong association between nicotine and alcohol use/abuse (Funk et al., 2006) has prompted researchers to explore the role of the nicotinic acetylcholine receptor (nAchR) in alcohol abuse and related neurocircuitry. The clinical literature suggests extraordinarily high rates of nicotine dependence in alcohol dependent subjects (Batel et al., 1995) and that the amount of alcohol and tobacco individuals consume are positively related (Barrett et al., 2006). Parallels have been observed in the animal literature. For example, a recent mouse study found that when nicotine was infused into the cholinergic-rich basal forebrain of male B6 mice, ethanol intake and BECs were increased ~40% in a standard DID paradigm (Sharma et al., 2014). An acute administration of ethanol can influence acetylcholine release in the PFC and hippocampus with lower doses increasing and higher doses inhibiting release (Henn et al., 1998; Stancampiano et al., 2004). Ethanol has also been shown to pharmacologically interact with nicotinic acetylcholine receptors (nAchRs), namely inhibiting homomeric α7 receptors and augmenting the activity of the α4β2 receptors (Davis and de Fiebre, 2006). A now well-known smoking cessation aid, varenicline, is a partial antagonist at α4β2 receptors and has been shown to reduce ethanol consumption in rats and mice (Hendrickson et al., 2010; Steensland et al., 2007), with some evidence of a similar effect in the clinical literature (McKee et al., 2009). One hypothesis for the high incidence of nicotine-ethanol co-abuse is that nicotine may enhance the effects of ethanol. Indeed, drug discrimination studies have shown that nicotine can increase the salience of low dose (0.5 g/kg) ethanol in B6 mice (Ford et al., 2012). Furthermore, nAch receptor activation may be a mechanism by which ethanol increases DA efflux in the accumbens (Ericson et al., 2008) and combined nicotine/ethanol have been shown to increase VTA DA neuron activity in an additive manner (Clark and Little, 2004). Indeed, an acute combined ethanol/nicotine injection produced additive locomotor stimulation in mice (Gubner et al., 2013), an effect associated with mesolimbic DA stimulation (Pijnenburg et al., 1976).

Pharmacological studies have explored how the regulation of nAchRs influences binge-like ethanol consumption. Systemic administration of the nonselective nAchR antagonist mecamylamine reduced saccharin and ethanol intake in 2-bottle DID (Ford et al., 2009).

However, Hendrickson et al. (2009) found systemic mecamylamine reduced DID ethanol, but not sucrose consumption in male B6 mice and that this effect was associated with reduced activaton of VTA DA neurons. Interestingly, systemic administration of nicotine and the alkaloids cytisine and lobeline, all with agonist properties at these receptors, reduced DID ethanol consumption (Hendrickson et al., 2010; Sajja and Rahman, 2011). These apparently disparate findings were interpreted as the ability of these agonists to mimic components of ethanol's effects on cholinergic signaling within the mesocorticolimbic pathway. Systemic varenicline also significantly reduced DID ethanol intake, an effect clearly mediated by the α4 subunit (Hendrickson et al., 2010). Furthermore, this effect was mirrored via local infusion of varenicline into the posterior, but not anterior VTA. Finally, Powers et al. (2013) found that greater ethanol intake via DID was associated with increased VTA DA neuron excitability in a genetic mouse model containing supersensitive α6 nAchR subunits.

These findings suggest that activation of DAergic neurons in the VTA via acetylcholine has the capacity to influence binge-like ethanol consumption and that the α4 subunit appears to play a particularly important role. Furthermore, nAchRs located in the basal forebrain also directly influence binge-like ethanol intake. Considering the additive potential of nicotine and ethanol to increase DA signaling in mesolimbocortical circuitry, an ample opportunity for microdialysis and/or electrophysiological experiments exists to explore additive neurochemical responses to binge co-consumption of nicotine and ethanol. An operant rat model of oral nicotine and ethanol co-consumption has been developed wherein P rats consume what could be considered binge levels of both compounds in a short period of 1 hr (Hauser et al., 2012).

Endogenous Opioid System

Ethanol has been demonstrated to increase the activity of the endogenous opioid system (Froehlich and Li, 1994). Within mesolimbocortical circuitry, ethanol's promotion of opioid receptor signaling has been hypothesized to inhibit GABA neurons located within the VTA, ultimately producing *disinhibition* of VTA DA neurons and increased DA release in the accumbens (Froehlich and Wand, 1996). Preclinical studies provide some evidence for this theory (Xiao et al., 2007) and a recent report demonstrated that although opioid receptor antagonists directly administered into the VTA did not prevent ethanol-induced DA efflux in the accumbens, it did reduce the duration of this effect (Valenta et al., 2013). There is also a translational connection between opioid signaling and ethanol abuse as the non-specific opioid receptor antagonist naltrexone, one of the few drugs approved to treat alcohol use disorders, significantly reduces ethanol intake in a variety of rodent models (O'Malley and Froehlich, 2002) and in heavy drinking human subjects (Spanagel and Vengeliene, 2013).

Naltrexone has been shown to reduce binge-like ethanol consumption in WSC male and female mice in a SHAC paradigm (Tanchuck et al., 2011) and in male B6 mice in a 2-hr DID procedure (Kamdar et al., 2007). Furthermore, a mouse strain exhibiting reduced expression and function of μ-opioid receptors (MORs) readily drinks ethanol in the standard DID paradigm and exhibits slightly reduced sensitivity to naltrexone-induced reductions in ethanol intake (Tarragón et al., 2012). These results suggest that the MOR may play a role in

binge-like alcohol consumption in mice, however the degree to which it influences binge ethanol intake, specifically, is not clear. Investigation of other opioid receptor subtypes has revealed an influential role for the δ subtype (DOR) as DOR KO mice demonstrate significantly greater binge-like ethanol intake relative to wildtype mice in 2-bottle DID (van Rijn and Whistler, 2009). Further exploration into the involvement of specific DOR receptor subtypes found that DOR1 activation reduces binge-like ethanol intake whereas DOR2 activation may promote consumption (van Rijn et al., 2010; van Rijn and Whistler, 2009).

Collectively, this work suggests that signaling through both MOR and DOR1, specifically, appear to be influential regulators of binge-like ethanol intake. The MOR has been shown to play an important role in ethanol consumption in other rodent drinking models (Hall et al., 2001; Lasek et al., 2007; Myers and Robinson, 1999; Roberts et al., 2000) with findings generally suggesting that MOR activation is highly important in the reinforcing and rewarding properties of ethanol. The findings of the one study addressing this question in a BD model (Tarragón et al., 2012) appear somewhat discordant with this body of literature, however it should be noted that the mice used in this study were an inbred cross of a B6 variant and BALB/cByJ and just happen to have a hypofunctioning MOR system. Therefore, more direct manipulation of MOR involvement in binge-like drinking is needed to clarify this relationship. DOR signaling has also been implicated in other models of ethanol self-administration as DOR KO mice will consume more ethanol in operant and 2-bottle choice paradigms with prolonged exposure (Roberts et al., 2001), however some pharmacological studies with non-selective DOR antagonists showed a decrease in intake (Franck et al., 1998; Froehlich et al., 1991). This highlights the importance of distinguishing between the DOR subtypes to explore their unique contributions as has been done by Van Rijn and colleagues (2009; 2010) whose work suggests that DOR2 may serve a promotional whereas DOR1 serves an inhibitory role in binge-like drinking. Finally, the involvement of the κ-opioid receptor (KOR) KOR in ethanol reward (Logrip et al., 2009) and 2-bottle choice drinking (Kovacs et al., 2005) has also been demonstrated, and this system has been implicated in chronic drinking or dependence models, having complex interactions with stress (Hölter et al., 2000; Sperling et al., 2010; Walker and Koob, 2008). However, the influence of the KOR in BD models is not known.

Corticotropin-releasing factor (CRF)

The involvement of CRF in stress responses has been known for some time. Indeed, activation of this system has been associated with increased ethanol responsivity and various aspects of the withdrawal state (Heilig and Koob, 2007). In addition, CRF receptor antagonists can selectively decrease ethanol intake in dependent, but not non-dependent animals, suggesting that this system is recruited in the transition to dependence (e.g. Funk et al., 2007). Acute ethanol exposure, however, increases the activity of CRF within hypothalamic neurons and can activate the hypothalamic-pituitary-adrenal axis (Li et al., 2005; Rivier and Lee, 1996). In addition, ethanol acts upon the CRF system to enhance GABAergic transmission in the central nucleus of the amygdala (CeA) of non-dependent animals (Nie et al., 2004), suggesting ethanol's effects on CRF signaling modify transmission in mesolimbocortical reward circuitry prior to the development of dependence.

A role for CRF in binge-like ethanol intake has been identified. CRF and CRF-1 KO mice demonstrate significantly reduced standard and 2-bottle DID ethanol consumption (Giardino and Ryabinin, 2013; Kaur et al., 2012). Pharmacological studies have also shown that CRF-1 receptor antagonism reduces standard and 2-bottle DID ethanol intake (Giardino and Ryabinin, 2013; Sparta et al., 2008), although Giardino and Ryabinin (2013) found that these effects were likely due to a non-specific effect on fluid intake, suggesting that methodological differences between DID paradigms may elicit different drug effects. Lowery and colleagues (2010) explored the role of central CRF receptors and found that intracerebroventricular infusion of a nonselective CRF receptor antagonist as well as a selective CRF-2 receptor agonist significantly reduced ethanol intake and BEC in a 4-hr DID procedure. Microinjections of a CRF-1 receptor antagonists directly into the CeA and VTA also significantly reduce DID ethanol intake (Lowery et al., 2010; Sparta et al., 2013). These observations are associated with an enhanced capacity of CRF to potentiate NMDA receptor signaling in the VTA and a reduced capacity for CRF to enhance GABAergic signaling in the CeA in mice with prolonged histories of DID ethanol consumption (Lowery-Gionta et al., 2012; Sparta et al., 2013). Urocortin 1 (Ucn1) is a neuropeptide similar in structure to CRF and binds readily to CRF1 and CRF2 receptors. Ryabinin and colleagues (2008) explored the role of Ucn1 and CRF within the lateral septum, an area known to be innervated by Ucn1-containing neurons, in binge-like ethanol intake. Intra-lateral septum Ucn1 was found to specifically reduce ethanol consumption, however CRF infusion reduced intake of water as well.

Collectively, this work suggests that repeated voluntary binge ethanol exposure recruits CRF mechanisms resembling those seen in dependence or chronic drinking models with both central and peripheral administration of CRF antagonists decreasing binge-like ethanol intake. This effect was not previously observed in non-dependent animals (Funk et al., 2007). In addition, binge-like drinking models demonstrated neuroplasticity of CRF signaling within mesolimbocortical circuitry related to both GABA and glutamate transmission. Finally, some early evidence suggests that Ucn1, also acting on CRF receptors, may exhibit a more specific effect than CRF on binge-like ethanol intake. Future studies should address the extent to which CRF effects may actually be explained by more specific Ucn1 signaling. Together, these observations raise questions about whether repeated BD may indeed recruit mechanisms implicated in dependence. Of course, the timeline of these potential effects and their relationship to behavioral phenotypes associated with dependence must be thoroughly explored.

Neuropeptide Y (NPY)

NPY has grown in interest in preclinical ethanol research due, in part, to its demonstrated effects on anxiety-like (Heilig et al., 1989) and appetitive behavior (Levine and Morley, 1984) in rodents. Interestingly, its activity appears to oppose that of CRF (Kash and Winder, 2006; Valdez and Koob, 2004) and NPY levels have been negatively associated with ethanol intake and sensitivity (Thiele et al., 1998). Similar to findings with CRF antagonists, central NPY administration reduces high alcohol intake of genetically predisposed high drinking animals and dependent animals with no effect on the alcohol intake of predisposed low drinking or non-dependent animals (Badia-Elder et al., 2003; Badia-Elder et al., 2001;

Gilpin et al., 2008). The two most well-known receptor subtypes, Y1 and Y2, appear to oppositely influence ethanol intake in rodents with Y1 agonism and Y2 antagonism decreasing ethanol intake across multiple rodent genotypes and drinking paradigms (Thiele et al., 2002; Thorsell et al., 2002). NPY is thought to reduce ethanol intake primarily through the Y1 receptor as these receptors are located postsynaptically, whereas Y2 receptors are presynaptic autoreceptors, activation of which inhibits endogenous NPY release (Sparrow et al., 2012).

Recognizing the association between CRF and NPY and previous observations of DID exposure influencing CRF neuroplasticity, the same research group similarly explored NPY's role in binge-like ethanol intake. In line with the findings of previous studies, Sparrow et al. (2012) found that central administration of NPY, a NPY1 receptor agonist, and a NPY2 receptor antagonist all significantly reduced binge-like ethanol intake. They also observed that a single 4-day cycle of DID significantly reduced NPY and NPY1 immunoreactivity in the CeA, and NPY was shown to enhance GABAergic inhibition only within the CeA of mice with a repeated DID history. Another recent study showed that Y2Rs located specifically on GABA neurons did not influence DID ethanol intake (McCall et al., 2013), indicating that the Y2R's effect on binge-like intake must be through a different neurotransmitter system.

Together, these results suggest that, similar to dependent or genetic animal models, central NPY signaling regulates binge-like drinking. Also in line with previous studies employing other drinking models, activation of Y1Rs and inhibition of Y2Rs reduced binge-like ethanol intake. Interestingly, NPY pharmacology was previously shown to have no influence on ethanol drinking below binge levels in mice (Thiele et al., 2003). Furthermore, the negative relationship between NPY levels and consumption (Thiele et al., 1998) was also supported as repeated cycles of binge-like drinking reduced NPY and the Y1R. Even so, the GABAergic response to NPY is potentiated by a history of binge ethanol consumption, perhaps suggesting increased functional properties of the receptor. Collectively, these findings corroborate the previously demonstrated link between CRF and NPY in ethanol intake and extend them to a model of BD.

Purinergic System

Ethanol is known to positively influence adenosine signaling indirectly via inhibition of its transporter, endonucleoside transporter 1, thereby increasing extracellular levels of adenosine (Nagy et al., 1990). This effect is thought to be partially responsible for the sedative response to ethanol intoxication (Choi et al., 2004), as adenosine signaling generally blunts neuronal excitability; an effect that may contribute to the extent of an individual's ethanol use. The two main adenosine receptor subtypes, A1 and A2a, have been implicated in ethanol-induced ataxia and sedation, respectively (Barwick and Dar, 1998; Connole et al., 2004; El Yacoubi et al., 2003; Naassila et al., 2002). Furthermore, the A2a receptor has been show to influence ethanol consumption in operant paradigms and 2-bottle choice drinking (Adams et al., 2008; Arolfo et al., 2004; Di Bonaventura et al., 2012; Thorsell et al., 2007), although the findings are currently equivocal. The authors are unaware of any study demonstrating an influence of the A_1 receptor on ethanol consumption.

Recently, our lab added caffeine, a non-selective adenosine receptor antagonist, to the 20% ethanol solution in DID at concentrations similar to that found in standard 'energy drinks.' Although the addition of caffeine had no effect on binge-like ethanol intake or BEC, the addition of caffeine significantly interfered with ethanol's depressant motor effects (Fritz et al., 2014a). However, caffeine co-consumption had no influence on ethanol-induced anxiolysis or memory interference. These results suggest that the motor, but not cognitive effects of voluntary binge ethanol intoxication may be more sensitive to adenosinergic regulation, a finding consistent with studies employing experimenter-administered caffeine and ethanol (López-Cruz et al., 2013). The role of specific adenosine receptor subtypes in binge-like drinking and its neurobehavioral consequences has not yet been studied.

Another form of purinergic signaling, P2X receptors are excitatory in nature by binding adenosine triphosphate (ATP) to promote cation flux. One particular subtype, P2X4, is particularly sensitive to inhibition via ethanol (Davies et al., 2005) and lower expression/ function has been linked to increased ethanol intake and preference (Kimpel et al., 2007; Tabakoff et al., 2009). Ivermectin, a widely-known antiparasitic drug, has been shown to be a positive allosteric modulator at these receptors. Yardley and colleagues (2012) found that systemic administration of ivermectin (10 mg/kg; i.p.) reduced ethanol intake by ~40% in a 4-hr DID paradigm. More recently, P2X4 KO mice exhibited increased 2-bottle choice DID ethanol intake and although ivermectin reduced their ethanol intake, it was 50% less effective than was demonstrated in wildtype mice (Wyatt et al., 2014).

The purinergic system is very diverse and clearly demonstrates sensitivity to binge levels of ethanol intake. The findings of these two studies illustrate that binge-like drinking and its motor effects are influenced by purinergic signaling, although far more work needs to be conducted to determine the role of adensosine receptor subtypes in these behaviors. Furthermore, the expression and functional characteristics of these receptors as a consequence of voluntary binge ethanol exposure should be addressed.

Other neurotransmitters

A number of studies have also explored the influence of the serotonin (Szumlinski et al., 2007a), endocannabinoid (Linsenbardt and Boehm II, 2009), ghrelin/leptin (Lyons et al., 2008), and melanocortin (Olney et al., 2014) systems in binge-like ethanol consumption. These findings are not reviewed here as there are relatively little data thus far and the roles of these systems in BD models are currently unclear.

Future Directions for the Field

Although our knowledge has clearly advanced substantially in recent years, much work remains to better understand the neurobehavioral mechanisms of BD and its consequences. Alternative research approaches will be necessary to effectively identify novel neurobiological pathways, and to more thoroughly characterize those already identified. A number of newer approaches hold much promise in this regard. We review several of them below.

Changes in gene (mRNA) expression are key to both normal and aberrant cellular function. Sophisticated molecular and statistical strategies now exist (e.g. microarray analysis) that 'cast a wide net' and allow for the identification of novel functional transcriptional pathways to guide future study. Several studies have used such approaches to probe for novel functional transcriptional pathways associated with binge-like drinking. In three of these studies, nucleus accumbens (Bell et al., 2009; Bell et al., 2006a), VTA (McBride et al., 2013), and CeA (Bell et al., 2006a) tissue from P rats that had been subjected to a DID-MSA procedure were analyzed. The expression of functional groups of genes related to neuroinflammation, steroid synthesis/degradation, cellular structure, and ethanol metabolism were all identified as being influenced by binge-like drinking. A fourth microarray study evaluated gene expression following a single 4-hr DID access period in male B6 mice (Mulligan et al., 2011). This group found that genes related to glutamate and serotonin signaling as well as blood circulation associated with astrocyte function were positively related to the BEC achieved in DID across numerous structures including the frontal cortex, striatum, ventral midbrain, hippocampus, cerebellum, and olfactory bulbs. Widespread screening of genes in this manner with more genetically heterogeneous animal models (i.e. HAP, HDID) may offer a powerful next step in focusing our efforts, especially if an analysis of BD duration influence is emphasized.

Molecular-based strategies have also been developed to assess epigenetic regulation of gene expression as a consequence of binge ethanol drinking. Focused on how environmental factors interact with genetic predispositions to produce a target phenotype, epigenetics is highly relevant to the field of substance abuse. One target in the search for epigenetic mechanisms associated with binge-like drinking is microRNAs (miRNAs). miRNAs expansively regulate downstream transcription of mRNA and are highly responsive to environmental factors such as drug or ethanol exposure (Li and van der Vaart, 2011) which may ultimately influence neuroplasticity and gene expression. Recently, Nunez and colleagues (2013) evaluated miRNA and associated mRNA transcriptional modifications in the frontal cortex of high drinking female B6 × FVB/NJ reciprocal cross mice following a 20 day 2-bottle choice DID procedure. miRNAs were indeed found to regulate transcription of target mRNAs with a high degree of consistency (particularly those involved in synaptic vesicle trafficking, endocytosis, and neuroimmune signaling). Collectively, the authors interpreted these modifications as a reflection of the system's attempt to achieve homeostasis in response to the effect of protracted binge ethanol consumption. There is ample opportunity for future studies to advance the field by addressing brain region-specific roles of miRNAs in the regulation of target proteins/processes as a consequence of various durations of voluntary binge ethanol consumption.

Recent 'state-of-the-art' *in vivo* experimental approaches have also been developed to more directly assess the intricate relationships between brain and behavior. As mentioned previously, current rodent BD models can produce pharmacologically relevant BECs and intoxication in a discrete period as short as 30 min. This is a key strength for highly precise techniques that require fine temporal resolution of behavior such as *in vivo* electrophysiology or highly-localized selective stimulation/inhibition of specific cell types via optogenetics (Aravanis et al., 2007). BD models provide readily definable periods of

drinking behavior which coincide well with event mapping in both techniques. Continuous access paradigms are simply not practical for such techniques due to both time requirements and concerns over pharmacologically relevant intake. Another elegant technique well-suited for these models is the designer receptors exclusively activated by designer drugs (DREADDS) approach (Lee et al., 2014). DREADDS technology essentially involves viral-vector mediated transfer of receptors designed to only respond to clozapine-*n*-oxide (CNO) and can be coupled to stimulatory or inhibitory G-proteins. A key advantage of this technique is that a simple systemic administration of CNO is all that is needed for the precise manipulation within the central nervous system, thereby allowing for a significant reduction in the complexity of data collection and analysis. These BD paradigms would be expected to produce intoxicating BECs in the discrete period of time where CNO would exert its designed pharmacological effect; something that could not be convincingly employed in a continuous access model. Therefore, these approaches should be considered in future preclinical BD research to explore its mechanistic underpinnings and consequences.

Finally, it may be obvious from the literature reviewed above that the current findings are largely restricted to a single rodent genotype (B6 mice) and the focus is mainly on male animals. Although the inbred B6 mouse strain is clearly the most readily available animal that will consume ethanol in a binge-like manner with virtually no training, researchers should consider HDID and HAP mice as alternative, and perhaps superior models as they also readily consume ethanol in a binge manner. These animals have been selectively bred for their respective ethanol consumption phenotypes which theoretically leaves unassociated alleles unfixed, making these mice substantially heterogeneous. In addition, it is becoming clearer that ethanol abuse is unique and perhaps more rapidly destructive in females (Greenfield et al., 2010; Keyes et al., 2010). Researchers should include both male and female animals in their experiments, and focused efforts should be made to understand how estrous cycle status uniquely alters binge-like intake in females.

In conclusion, the field of preclinical BD research has revealed many exciting neurobiological targets for further study. As efforts are focused to advance our understanding in the areas mentioned above, it will be important to explore the mechanisms of binge-like drinking over varying durations of exposure, offering insight on the progression of BD and whether it may lead towards dependence. With the current mechanistic/genetic techniques available to researchers today, we encourage the use of these convenient and reliable rodent BD models which offer substantial potential to rapidly progress our understanding of the biomolecular underpinnings and consequences of BD.

Acknowledgments

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Abbreviations

BD   binge drinking

BEC   blood ethanol concentration

CNO   clozapine-n-oxide

B6   C57BL/6J inbred mice

CRF   Corticotropin-releasing factor

DOR   δ-opioid receptor

DREADDS	designer receptors exclusively activated by designer drugs
DA	dopamine
DAT	dopamine transporter
DID	Drinking-in-the-Dark
DID-MSA	Drinking-in-the-Dark - multi-scheduled-access
GABA	γ-aminobutyric acid
HAP	high alcohol-preferring mice
HARF	High Alcohol Research Foundation rats
HDID	High-drinking-in-the-dark mice
KOR	κ-opioid receptor
KO	knockout
D2LR	long dopamine D2 receptor isoform
LARF	Low Alcohol Research Foundation rats
miRNAs	microRNAs
MOR	μ-opioid receptor
nAchR	nicotinic acetylcholine receptor
NMDA	N-methyl-D-aspartate
MOR	μ-opioid receptor
NPY	Neuropeptide Y
SHAC	scheduled high alcohol consumption
D2LR	short dopamine D2 receptor isoform
VTA	ventral tegmental area