Involvement of activated brain stress responsive systems in excessive and “relapse” alcohol drinking in rodent models: implications for therapeutics

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List of Abbreviations: ADE, alcohol deprivation effect; AEA, anandamide; AVP, arginine vasopressin; BNST, bed nucleus of the stria terminalis; CB, cannabinoid receptors; CeA, central nucleus of amygdala; CNS, central nervous system; CPP, conditioned place preference; CRF, corticotrophin releasing factor; DGAVP, desglycinamide-(Arg^8)-vasopressin; DID, drinking-in-the-dark; eCB, endocannabinoids; eGFP, enhanced green fluorescent protein; FAAH, fatty acid amide hydrolase; HPA, hypothalamic-pituitary-adrenal axis; IA, intermittent access drinking; KO, knockout; KOP-r, kappa-opioid receptor; MC4, melanocortin 4 receptor; MOP-r, mu-opioid receptor; MSB, mesyl salvinorin B; NAc, nucleus accumbens; nor-BNI, nor-binaltorphimine; nPE, neuronal Pomc enhancers; NTN, naltrexone; POMC, pro-opiomelanocortin; PVN, paraventricular nucleus; sNP, Sardinian alcohol-non preferring; sP, Sardinian alcohol-preferring rats.

Recommended section: Neuropharmacology
Abstract

Addictive diseases, including addiction to alcohol pose massive public health costs. Addiction is a chronic relapsing disease, caused by both the direct effects induced by drugs and persistent neuroadaptations at several (molecular, cellular and behavioral) levels. These drug-type specific neuro-adaptations are brought on largely by the reinforcing effects of drugs on the central nervous system and environment, including stress. Results from animal experiments have demonstrated important interactions between alcohol and the stress responsive systems. Addiction to specific drugs (such as alcohol, psychostimulants and opioids) shares some common direct or downstream effects on the brain stress-responsive systems, including arginine vasopressin and its V1b receptors, dynorphin and the kappa opioid receptors, pro-opiomelanocortin/β-endorphin and the mu opioid receptors, and the endocannabinoids. Further study of these systems, through laboratory-based and translational research, could lead to the discovery of novel treatment targets and the early optimization of interventions (for example, combination) for the pharmacological therapy of alcoholism.
Introduction

An expanding literature demonstrates that alcohol activates the brain stress responsive systems, which contribute to excessive alcohol drinking, and the development of alcoholism with relapse of alcohol use. There are several recent reviews from 2016-2018 providing the details of other important stress responsive systems (like Corticotrophin Releasing Factor, Neuropeptide Y, and glucocorticoid receptor, etc.) from preclinical evidence to the recent clinical trials [Koob and Mason, 2016; Mantsch et al., 2016; Blaine and Sinha, 2017; Mason, 2017; Robinson and Thiele, 2017; Spierling and Zorrilla, 2017; Tunstall et al., 2017; Pomrenze et al., 2017]. The main focus in this mini-review is several other important stress responsive systems, which recently has not been reviewed, like arginine vasopressin/V1b receptors (Part I), and pro-opiomelanocortin/β-endorphin (Part II). Also, in other two stress responsive systems, endocannabinoids/fatty acid amide hydrolase [Part III], and dynorphin/kappa opioid receptors [Part IV], we examine possible explanations for the controversy in the literature, to assess the most current state of the field [Parsons and Hurd, 2015; Chavkin and Koob, 2016; Anderson and Becker, 2017; Karkhanis et al., 2017; Tunstall et al., 2017]. Therefore, this mini-review will provide an overview of the recent literature on the above four stress responsive systems in alcohol research, using laboratory based animal models and clinical research to elucidate the biology of addictive diseases. We propose that the translational bi-directional research will help refine future preclinical targets for pharmacological therapy of alcoholism.

I. V1b receptor and arginine vasopressin (AVP) system

In the neurobiology of stress-related behaviors, increased AVP neuronal activity is involved in important steps in several rodent models [Griibel et al., 2002; Salome et al., 2006; Roper et al., 2011] and in humans [Katz et al., 2016; Ryan et al., 2017]. Since the 1980’s, evidence emerged indicating AVP in the motivational properties of drugs of abuse [see van Ree et al., 1999]. In studies exploring the role of AVP in rhesus monkeys, systemic administration of
desglycinamide-(Arg⁸)-vasopressin [DGAVP] reduces alcohol intake [Kornet et al., 1991]. DGAVP also is found to decrease alcohol intake in Brattleboro homozygote rats lacking vasopressin [Rigter and Crabbe, 1985] (Table 1). Of interest, after chronic exposure to an alcohol-containing diet, AVP mRNA levels are found to be decreased in several stress-responsive brain regions of C57BL/6J mice, including the paraventricular nucleus (PVN), supraoptic nucleus and bed nucleus of the stria terminalis (BNST) [Ishizawa et al., 1990; Hoffman, 1991; Gulya et al., 1991]. Further studies demonstrate a decrease of the number of AVP-immunoreactive neurons and reduction of AVP mRNA levels in the hypothalamus after chronic alcohol consumption in both humans [Harding et al., 1996] and rats [Silva et al., 2002]. In humans, there are abnormal levels of serum and urine AVP during alcohol withdrawal, particularly when symptoms are severe [Eisenhofer et al., 1985; Trabert et al., 1992].

In several selectively bred alcohol drinking rat lines, there are higher basal levels of AVP mRNA in the PVN of Indiana alcohol-preferring rats, Sardinian alcohol-preferring (sP) rats and high-alcohol-drinking rats, as compared with their alcohol-non preferring and low-alcohol-drinking counterparts, respectively [Hwang et al., 1998; Zhou et al., 2011a]. Higher basal AVP mRNA levels are also found in the medial amygdala of sP rats than Sardinian alcohol-non preferring (sNP) rats; and chronic (more than 2 weeks) alcohol drinking reduces the AVP mRNA levels in the PVN and medial amygdala of sP rats [Zhou et al., 2011a]. Of interest, individual differences in AVP mRNA levels are observed to positively associate with vulnerability to high alcohol drinking in C57BL/6J mice after acute stress [Nelson et al., 2018]. As individual vulnerability to drug relapse during abstinence is a key feature of drug addiction, more studies are really needed [Imperio et al., 2016; Sushchyn et al., 2016].

AVP binds to two G protein-coupled receptor subtypes in the brain: V1a and V1b and both are expressed in the extended amygdala, with high concentrations in the central nucleus of amygdala (CeA), the BNST and nucleus accumbens (NAc) [Veinante and Freund-Mercier 1997]. Specifically, V1b receptor is mostly distributed in the PVN, hippocampus, and amygdala,
as well as the anterior pituitary [Lolait et al., 1995; Vaccari et al., 1998; Hernando et al., 2001; Young et al., 2006]. In rodent models, many studies suggest that augmented AVP/V1b activity in the amygdala plays a critical step in the stress-related behaviors: [1] after acute stress, there is an increased extracellular AVP level in the rat amygdala [Wigger et al., 2004]; [2] an increase of AVP mRNA levels in the amygdala was observed after acute withdrawal stress from drug exposure or by foot-shock stress in the rats after drug self-administration [Zhou et al., 2005, 2008]; and [3] activation of V1b receptors is involved in anxiety-like and depression-like behaviors [Griebel et al., 2002; Serradeil-Le Gal et al., 2002; Salome et al., 2006; Roper et al., 2011]. SSR149415 (a highly selective non-peptide antagonist for the V1b receptor), has anxiolytic-like and antidepressant-like properties [e.g., Overstreet and Griebel 2005].

One of the critical factors influencing individual vulnerability to drug relapse is atypical stress responsivity [Kreek and Koob, 1998; Zhou and Kreek, 2014]. Using many validated experimental models, like the forced swim and elevated plus maze tests in rodents, anxiety-like and depression-like behaviors have been demonstrated after chronic alcohol, mostly during its acute withdrawal [Colombo et al., 2006; Bell et al., 2012; Becker and Happel, 2012]. As the high degree of anxiety-like and depression-like states is partially attenuated after voluntary alcohol drinking, rodents may drink alcohol to improve their emotional states (negative reinforcing mechanism) [Colombo et al., 2006; Bell et al., 2012; Pang et al., 2013]. In our alcohol study with sP rats, V1b antagonist SSR149415 dose-dependently attenuates alcohol intake, suggesting that V1b receptor-mediated mechanism is involved in modulating alcohol drinking behaviors [Zhou et al., 2011a]. Importantly, SSR149415 reduces excessive alcohol self-administration in alcohol-dependent Wistar rats in a dose-dependent manner, without altering alcohol drinking in non-dependent rats [Edwards et al., 2012] (Table 1). Systemic administration of V1b antagonists blocks the stress- and drug priming-triggered seeking behavior [Zhou et al., 2008] and prevents the dysphoria induced by nicotine withdrawal [Qi et al., 2015], as well as nicotine-induced locomotor sensitization [Goutier et al., 2016]. Therefore, the AVP/V1b system is a
critical component contributing to the negative reinforcing effects of alcohol, heroin or nicotine, especially during drug withdrawal. A recent phase two, double-blind clinical trial (Table 1) finds that pharmacological blockade of the V1b receptor reduces alcohol consumption and relapse rate in alcohol-dependent patients, especially those with high stress [Ryan et al., 2017]. As the V1b receptor is a feasible target in humans, there is translational potential for novel anti-alcoholism medications.

Stress increases secretion of corticotrophin releasing factor (CRF) and AVP (the parvocellular division of the PVN) from terminals of the PVN into the pituitary portal circulation. The interaction between CRF and CRF1 receptors on corticotropes initiates the biosynthesis of pro-opiomelanocortin (POMC)-derived peptides and their release from the anterior pituitary [Vale et al., 1981]. AVP from the parvocellular division of the PVN activating V1b receptors in the corticotropes enhances ACTH secretion from the anterior pituitary [Lolait et al., 1995; Aguilera and Rabadan-Diehl, 2000]. However, AVP neurons in the magnocellular division of the PVN project to the posterior pituitary and then release AVP into the systemic circulation in response to stress. Both CRF/CRF1 receptor and AVP/V1b receptor systems are also mediators of the actions of central stress responsive systems, as both are widely distributed in the CNS [Zhou et al., 1996; Roper et al., 2011]. Different from the hypothalamic CRF in response to acute cocaine, several studies have shown that AVP in the PVN does not contribute to the acute stimulatory effects of alcohol on HPA activity [Rivier and Vale, 1988; Lee and Rivier, 1997]. It is still not known, however, if the AVP/V1b receptor systems are specifically involved in the HPA modulation during acute or chronic withdrawal from alcohol exposure or after relapse-like drinking in rodent models, though AVP is potent modulator of HPA axis. While the activation of the PVN CRF contributes to the stimulating effect of acute alcohol on the HPA axis [e.g., Rivier and Vale, 1988], chronic alcohol exposure blunts HPA hormonal response to alcohol, showing the development of HPA tolerance with either no change or even decreased CRF mRNA level in the PVN [Zhou et al., 2000; Richardson et al., 2008]. Acute and protracted
withdrawal form alcohol is coupled with decreased levels of both plasma corticosterone and hypothalamic CRF-like immunoreactivity in alcohol dependent rats [Zorrilla et al., 2001]. In humans, acute exposure to alcohol profoundly activates the HPA axis, and many alcoholics develop HPA tolerance after chronic alcohol exposure [Adinoff et al., 1990; Inder et al., 1995]. In contrast, acute alcohol withdrawal transiently activates HPA axis [Hundt et al., 2001; Zimmermann et al., 2003]. Also, the noradrenergic system, the known key stress mediator that is involved in stress and anxiety responses as demonstrated before [e.g., Tunstall et al., 2017], probably interacts with AVP and CRF systems to regulate alcohol drinking and HPA activity.

II. Pro-opiomelanocortin (POMC)/β-endorphin and mu-opioid receptor (MOP-r) system

In the pituitary, ACTH is produced from the anterior lobe corticotrophs, whereas N-acetylated forms of β-endorphin and α-melanocyte-stimulating hormone are produced in the intermediate lobe melanotrophs. In the brain, the arcuate nucleus of the hypothalamus processes POMC to produce the potent opioid peptide β-endorphin, and α-, β-, and γ-melanocortins [Ragavan et al., 1983; Rubinstein et al., 1996; Cowley et al., 2001; Romanova et al., 2015]. Besides the arcuate nucleus, POMC mRNA molecule has also been detected in several other mouse and rat brain regions, including the NAc, amygdala, hippocampus and cerebral cortex, in much lower levels than those in the arcuate nucleus [Civelli et al., 1982; Zhou et al., 1996, 2013a; Leriche et al., 2007; Bodnar, 2014; Granholm et al., 2017]. Using the POMC-eGFP mice, we observe that POMC expression in POMC-eGFP neurons can be visualized by GFP immunohistochemistry, and that there is a modest amount of POMC-eGFP neurons present in both the shell and core sub-regions of NAc [Zhou et al., 2013a]. In the NAc, the amount of POMC mRNA is ~ 10% of that detected in the hypothalamus, and the relative low POMC mRNA signal in the NAc is correlated with the relatively small number of POMC-eGFP neurons in the POMC-eGFP mice. Though alcohol drinking for more than 2 weeks increased POMC mRNA in the NAc shell (but not core) of sP rats, it remains unclear whether the POMC
mRNA in the extra-hypothalamic regions (e.g., NAc shell and core) will be processed to melanocortins, β-endorphin or other functional peptides.

In the rat anterior pituitary, an increase, a decrease or no change of POMC mRNA levels, as well as the levels of POMC-derived peptides, has been reported after acute or chronic alcohol administration [Gianoulakis et al., 1988; Dave et al., 1986; Winkler et al., 1995; Zhou et al., 2000, 2013a]. Using pituitary-specific deletion of POMC gene in Tpit transgenic mice, our recent study found that pituitary POMC deficiency does not change either alcohol drinking in a drinking-in-the-dark (DID) model (with 4-hour limited access to alcohol in the dark cycle) or alcohol-induced conditioned place preference (CPP) in male or female mice, suggesting that the pituitary POMC cells may not involve in the rewarding action or “binge” consumption of alcohol [Zhou et al., 2017b].

In the hypothalamus, POMC mRNA levels are either increased or decreased after acute or chronic alcohol [Angelogianni and Gianoulakis, 1993; Zhou et al., 2000, 2013a; Rasmussen et al., 2002; Navarro et al., 2013]. The C57BL/6 mice have high basal POMC mRNA levels in the hypothalamus, with high alcohol intake or preference, as compared with the alcohol-avoiding DBA/2 mice with low alcohol intake or preference [Jamensky and Gianoulakis1999]. In parallel, we have found that the sP rats have higher basal POMC mRNA levels in the hypothalamus than the sNP rats, and chronic alcohol drinking for more than 2 weeks results in further increases in the hypothalamic POMC mRNA levels in the sP rats [Zhou et al., 2013a]. Considering the well-established role of β-endorphin in alcohol drinking behaviours, the high alcohol preference and/or consumption found in sP rats and C57BL/6J mice may be attributed by the above genetically-determined POMC expression at basal levels and in response to alcohol.

Activation of MOP-r by β-endorphin produces rewarding [Barson et al., 2011; Koch et al., 2015] and regulates NAc dopamine release [e.g., Spanagel et al., 1991]. Alcohol or other drugs of abuse may release β-endorphin in the NAc [Olive et al., 2001; Marinelli et al., 2003; Roth-Deri
et al., 2008], and the effects could be involved in the reinforcing actions and motivational behaviors of the drugs of abuse in rodents. Indeed, icv administration of β-endorphin induces CPP in rats [Amalric et al., 1987]. Consistent evidence has been provided by numerous pharmacological studies in rodents showing that opioid antagonists reduce alcohol consumption, reward, reinstatement of seeking behavior induced by cue, and “relapse” drinking. In human alcoholics, the opioid antagonist naltrexone decreases alcohol drinking, craving and relapse [e.g., Brown and Holtzman, 1981; Hall et al., 2001; Liu and Weiss, 2002; Kuzmin et al., 2003; Pastor et al., 2011; Lukas et al., 2013] [see reviews by Gianoulakis, 1993; Heinz, 1997; Le Merrer et al., 2009]. In MOP-r knockout mice, there is a decrease in alcohol drinking or self-administration [Roberts et al., 2000; Hall et al., 2001; Ben Hamida et al., 2018] (Table 2), further indicating that the β-endorphin/MOP-r plays a functional role in the modulation of alcohol drinking.

POMC neurons in the hypothalamus, the main region producing β-endorphin in the brain, may contribute to alcohol consumption. It was not clear, however, whether there is an involvement of β-endorphin in regulation of alcohol drinking, as earlier studies using β-endorphin deficient mice show inconsistent results by different groups [Grisel et al., 1999; Grahame et al., 2000; Racz et al., 2008] (Table 2). A limitation of this global beta-endorphin knockout mouse model is that it does not allow for clarification of which specific regions of POMC cells (e.g., hypothalamus or possible pituitary) are involved in alcohol drinking behaviors. Recently, neuronal Pomc enhancers (nPE1 and nPE2) that are necessary for POMC expression specifically in hypothalamic arcuate neurons have been identified. Simultaneous transcriptional interference of Pomc enhancers function by insertion of a neomycin selection cassette in the enhancer vicinity abolishes POMC gene expression in hypothalamic arcuate nucleus of transgenic mice, while leaving normal levels of POMC expression in pituitary cells [Bumaschny et al., 2012]. Therefore, to determine the role of hypothalamic POMC neurons in alcohol drinking behaviors, we have recently used transgenic mice with region-specific POMC
deficiency resulting from selective deletion of Pomc enhancers [Lam et al., 2015]. Specifically, we determine the effect of tissue-specific Pomc gene manipulation on: (a) “binge” drinking in a DID model [Rhodes et al., 2005]; (b) acquisition and escalation of excessive alcohol drinking in a chronic intermittent access (IA) model [Wise, 1973; Simms et al., 2008; Hwa et al., 2011], and (c) “relapse” drinking in an alcohol deprivation effect (ADE) model in mice of both sexes [Holter and Spanagel, 1999; Heyser et al., 2003]. The wild-type mice exposed to DID rapidly establish stable alcohol drinking behavior with more intake in females, whereas hypothalamic POMC deficient mice of both sexes have less alcohol intake and less preference. Though hypothalamic POMC deficient mice show less saccharin intake and preference than the wildtypes, there is no genotype difference in sucrose intake or preference. After 3-week IA, the wildtype mice gradually escalate to high alcohol intake and preference, with more intakes in females, whereas the hypothalamic POMC deficient mice show less escalation. Of interest, pharmacological blockade of MOP-r with naltrexone (NTN) dose-dependently reduces intake in the wildtypes, but has blunted effects in the hypothalamic POMC deficient mice. The wildtype of both sexes displays significant relapse-like ADE drinking, with more pronounced ADE in females, whereas the hypothalamic POMC deficient mice do not show ADE in either sex. Together, our results suggest an involvement of neuronal POMC/β-endorphin in regulation of “binge” drinking, excessive drinking and “relapse”, possibly through a hypothalamic-mediated mechanism, with sex differences [Zhou et al., 2017b] (Table 2). Consistently, mice lacking MOP-r show reduced excessive alcohol drinking [Ben Hamida et al., 2018].

Consistent with previous studies in mice [Hall et al., 2001; Racz et al., 2008; Hwa et al., 2011; Yoo et al., 2012] and rats (recently reviewed by Becker and Koob 2016), we confirm sex differences in alcohol drinking, with higher alcohol intake in females. The genotype differences in alcohol intake between hypothalamic POMC deficient and wildtype mice are much greater in females than in males. The POMC deficiency affects female mice more strongly than males, suggesting that POMC may influence alcohol consumption in a sex-specific manner [Zhou et al.,
2017b]. Our result is in line with early studies demonstrating a decreased alcohol intake in beta-endorphin and MOP-r knockout mice with more notable differences in females [Hall et al., 2001; Racz et al., 2008] (Table 2). Sex differences have also been observed in a human genetic study, showing that the Pomc two-marker haplotype is associated with alcoholism only in women [Racz et al., 2008]. These results also contribute to the idea that there are sex differences in opioid regulation of alcohol dependence [Becker and Koob, 2016].

Activation of POMC neurons affects food intake (which is increased and decreased by endorphin and melanocortins, respectively) especially at the onset of the dark cycle in mice [Mercer et al., 2013], so we purposely monitor drinking activity in the IA model during 24-hour cycle with 3 timepoints: the first 4-hour dark cycle, the 2nd 4-hour dark cycle, and the whole light cycle. Both male and female mice display escalated alcohol intake after 3 weeks of chronic IA exposure, mainly occurring at the first 4-hour dark cycle (25-30% in total daily intake), without much change in the other two-time periods [Zhou et al., 2017a, b]. Of interest, hypothalamic POMC deficient mice displayed lower alcohol intake than wild-type mice during the first 4-hour dark cycle in both sexes [Zhou et al., 2017b], suggesting a potential contribution of hypothalamic POMC to the genetically determined tendency of hypothalamic POMC deficient mice towards reduced alcohol consumption, with potential clock genes’ influence, as found in other studies with alcohol [Spanagel et al., 2005; Agapito et al., 2010; Partonen, 2015].

β-Endorphin is critically involved in the regulation of HPA activity. In both animal and human studies, it has been demonstrated that endogenous β-endorphin has tonic inhibition of the HPA axis by acting on the MOP-r [e.g., Kreek and Koob, 1998; Wand et al., 2002; Zhou et al., 2017b]. NTN is a clinical MOP-r antagonist in the treatment of alcoholism [O’Malley et al., 1992; Volpicelli et al., 1992]. As β-endorphin exerts tonic inhibition of CRF in the PVN (central part of the HPA axis), NTN blocks MOP-r, disinhibits the inhibition of the CRF and then acutely and persistently activates the HPA axis [O’Malley et al., 2002]. The NTN-treated group shows higher plasma ACTH and cortisol levels than the placebo-treated group in this human study. Of
great interest, alcohol craving levels in both groups are negatively correlated with plasma cortisol levels. As the first human laboratory study, the results clearly demonstrate that either the suppression of alcohol craving or the reduction in alcohol drinking may be contributed by the modest activation of the HPA axis by NTN [O’Malley et al., 2002]. Support for this finding can be also found in other human studies by other groups [e.g., Schuckit, 1994].

The potential roles of endogenous ACTH and melanocortins in the brain (encoded by the Pomc gene) in regulation of alcohol-related behavior is not clear. Recent pharmacological studies, have demonstrated that specific melanocortin 4 receptors (MC4) agonists significantly decrease alcohol “binge”-like drinking in a DID model, as well as reduce appetitive and consumption behaviors [Olney et al., 2014; Sprow et al., 2016]. In contrast, another recent report has found that MC4 receptor antagonists in the ventral tegmental area reduce alcohol self-administration in rats [Shelkar et al., 2015], suggesting that endogenous melanocortins and MC4 activation mediate the alcohol-reinforcing effect.

**III. Endocannabinoid system**

The endocannabinoid (eCB) system contains endogenous cannabinoids (including anandamide [AEA] and 2-arachidonoyl glycerol) and cannabinoid receptors (CB1 and CB2). In rodents, pharmacological studies have demonstrated that specific blockade of CB1 receptors decreases alcohol drinking, blocks the motivation to consume alcohol and reduces alcohol seeking, suggesting that the eCB/CB1 system is important in mediating the positive reinforcing properties and consumption of alcohol [Arnone et al., 1997; Colombo et al., 1998; Gallate and McGregor, 1999; McGregor et al., 2005]. Furthermore, CB1 knockout mice show reduced alcohol drinking or preference and alcohol reward [Hungund et al., 2003; Wang et al., 2003; Naassila et al., 2004; Houchi et al., 2005]. Therefore, during early stages of alcohol drinking, the increased eCB/CB1 activity may promote alcohol reward and then enhance alcohol intake [Manzanares et al., 1999]. After chronic alcohol exposure and protracted withdrawal, however,
there may be an eCB/CB1 signaling deficiency, which could also increase alcohol intake via the negative reinforcement mechanism. This notion is supported by several findings: [1] there is down-regulation of CB1 expression and function observed during protracted alcohol withdrawal in rats [Mitrirattanakul et al., 2007; Varodayan et al., 2016]; [2] in human imaging studies, decreased CB1 availability is observed in heavy-drinking alcoholics that persists into abstinence [Hirvonen et al., 2013; Ceccarini et al., 2014]; and [3] a lowered plasma AEA level is found in alcohol dependent patients during recent abstinence [Mangieri et al., 2009].

The AEA-dependent signaling is regulated by an enzyme involved in AEA catabolism, fatty acid amide hydrolase (FAAH) [Cravatt et al., 1996, 2001]. Numerous studies have demonstrated that there is involvement of AEA in the behavioral effects of alcohol. In FAAH knockout mice, there is a resultant increase in AEA levels [Cravatt et al., 2001], and the FAAH knockout mice show increased alcohol consumption and preference [Basavarajappa et al., 2006; Blednov et al., 2007] (Table 3). In human genetic studies, increased alcohol abuse and dependency are associated with the FAAH C385A polymorphism (increased eCB activity due to impaired FAAH function) [e.g., Sipe et al., 2002; Sloan et al., 2018] (Table 3). Consistently, we found that there is increased alcohol consumption in the knock-in mice with the human FAAH C385A [Zhou et al., 2016], with reduced anxiety-like behavior [Dincheva et al., 2015].

Increased stress responsivity and persistent negative affective symptoms, such as anxiety and depression, are observed during alcohol withdrawal, and the severity may be associated with alcohol relapse susceptibility [Koob and Kreek, 2007]. eCBs have considerable modulatory effects on the extended amygdala and corticostriatal circuitries, and stress disrupts these eCB-enriched regions that are involved in emotional control [Serrano et al., 2012; Dincheva et al., 2015; Morena et al., 2016]. Pharmacologic and genetic manipulations (knockout or knock-in) of FAAH are found to alter anxiety-like and depression-like behaviors [Bortolato et al., 2007; Gunduz-Cinar et al., 2013; Kathuria et al., 2003; Moreira et al., 2008; Carnevali et al., 2015]. Therefore, increased anxiety and depression are associated with the relatively deficient...
eCB function. Thus, the negative affective states and increased stress responsivity that underlie negative reinforcement mechanisms driving alcohol drinking by dependent individuals may be contributed by impaired eCB activity, which may also contribute to alcohol relapse following abstinence [Parsons and Hurd, 2015].

Though FAAH inhibition is found to decrease anxiety-like behaviors that are present during alcohol withdrawal [Cippitelli et al., 2008], there was no study to test the effect of FAAH inhibitors on alcohol drinking during withdrawal. We recently hypothesize that FAAH inhibition will enhance eCB signaling and then reduce the negative effect of alcohol withdrawal, which may reduce excessive and “relapse” drinking. Therefore, our recent study investigates whether URB597 (a selective FAAH inhibitor) would alter alcohol drinking in mice during acute or chronic withdrawal from 3-week chronic IA excessive alcohol drinking, to explore its potential for its therapeutic agent for alcoholism [Zhou et al., 2017c]. We also investigate the pharmacological effects of URB597 (clinical FAAH inhibitor) on the ADE, in which mice are allowed to access to alcohol after 1 week of abstinence. After acute withdrawal from chronic IA, pretreatment with URB597 reduces alcohol intake and preference in both male and female mice. This effect is mediated through CB1 receptor. Of interest, the ADE can be prevented by either single- or multiple-dosing regimen with an effective dose of URB597, with no tolerance after 1 week of the multi-dosing regimen. At the most effective dose for reducing alcohol intake, URB597 has no effect on sucrose or saccharin preference in alcohol naïve mice, but increases sucrose preference in mice after alcohol withdrawal [Zhou et al., 2017c]. In previous work, URB597 is found to increase sucrose preference in stress-exposed animals, probably due to its “anti-depression” properties [Rademacher and Hillard, 2007; Bortolato et al., 2007]. Consistent with studies on cocaine, nicotine and opioid seeking behaviour [Panlilio et al., 2013; Sloan et al., 2017], our findings show initial, promising data that FAAH inhibitors decrease alcohol excessive drinking, and “relapse” drinking in both male and female mice. Consistently, a new report demonstrates that the CeA of alcohol-preferring rats is involved in the URB597 effect on...
reducing alcohol drinking [Stopponi et al., 2018] (Table 3). Together, these results clearly suggest that the inhibition of FAAH plays a critical role in regulating alcohol drinking and related behaviors. Therefore, FAAH inhibitors, with improved pharmacokinetics (long-lasting in vivo bioactivity, e.g., URB597) [Fegley et al., 2005; Basavarajappa et al., 2014] and with no rewarding effect [Gobbi et al., 2005], have the potential to become useful compounds for treating alcoholism [Zhou et al., 2017c; Stopponi et al., 2018].

IV. Kappa opioid receptor (KOP-r) and dynorphin system

Activation of the KOP-r/dynorphin system is involved in aversive, dysphoria-like and depression-like behaviors. For example, aversive behaviors triggered by repeated forced swim or foot-shock stress are blocked by KOP-r antagonists or absent in dynorphin knockout mice [Land et al., 2008]. Further study using an opto-genetic approach has demonstrated that the dynorphin/KOP-r in the NAc shell plays a function role in aversive behaviors [Al-Hasani et al., 2015]. The dysphoric properties of chronic stress are encoded by dynorphin acting on KOP-r in specific stress-related brain regions, as the dynorphin-dependent KOP-r activation by stress is found in these brain regions (including the basolateral amygdala, NAc, dorsal raphe, and hippocampus). Together, dynorphin/KOP-r system is a key mediator of stress induced aversion, dysphoria, and anxiety- and depression-like behaviors [Butelman et al., 2012; Lalanne et al., 2014]. Like stressors, KOP-r agonists stimulate HPA activity in rats, and selective KOP-r antagonist nor-BNI blocks the stimulatory effects of the KOP-r agonists on the HPA axis [e.g., Laorden and Milanes, 2000; Pascoe et al., 2008]. Consistently with the early evidence that KOP-r/dynorphin regulates the HPA axis, we confirm that blockade of KOP-r with nor-BNI prevents ACTH and corticosterone increases induced by acute stress in our recent studies [Allen et al., 2013; Zhou et al., 2013b]. In humans, KOP-r agonists or partial agonists increase plasma ACTH and cortisol levels [Ur et al., 1997; Schluger et al., 1998], and short-acting KOP-r antagonist (LY2456302) does not cause aversive effects or HPA activity [Reed et al., 2018].
KOP-r/dynorphin activation is associated with the negative reinforcement aspects of alcohol addictions. It has been found that selective blockade of KOP-r attenuates excessive drinking, stress or cue-induced alcohol-seeking in mice and rats [Walker and Koob, 2008; Sperling et al., 2010; Deehan et al., 2012; Schank et al., 2012; Funk et al., 2014; Rorick-Kehn et al., 2014; Anderson et al., 2016; Zhou et al., 2017a] (but also see Mitchell et al., 2005; Sirohi et al., 2016). In line with these pharmacological results, alcohol drinking is decreased in KOP-r knockout mice [Kovacs et al., 2005]. These findings provide support for the critical involvement of the KOP-r/dynorphin system in the process of alcohol addiction, though the literature is not consistent. There are sex differences in dynorphin/KOP-r systems [Chartoff and Mavrikaki, 2015] and alcohol drinking behavior [Becker and Koob, 2016]. Indeed, we observe a reduction of alcohol drinking with the selective KOP-r antagonist nor-binaltorphimine (nor-BNI, slow onset and extraordinarily long-lasting effect [Horan et al., 1992]) in male mice, while the same nor-BNI treatment has no effect on alcohol drinking in female mice [Zhou et al., 2017a].

Micro-dialysis studies have demonstrated that acute alcohol increases the extracellular levels of dynorphin A1-8 in the CeA and NAc, two brain regions known to play important roles in the regulation of alcohol consumption [Marinelli et al., 2006; Lam and Gianoulakis, 2011]. Dynorphin mRNA levels in the CeA are found to be increased in the rats after acute alcohol withdrawal from multiple “binge” administrations of alcohol [D’Addario et al., 2013]. The CeA is one of critical brain regions mediating depression-like and anxiety-like behaviors [Shippenberg et al., 2007; Knoll and Carlezon, 2010], and is a possible site for the potential interaction of alcohol and the KOP-r/dynorphin. In fact, in sP rats after large amount of alcohol drinking, an increase in dynorphin mRNA levels is found in the CeA. Therefore, the KOP-r/dynorphin involved in neuronal structures related to stress responsivity (e.g., CeA) is activated after high levels of alcohol consumption in sP rats [Zhou et al., 2013c]. It is further confirmed that there are increases in dynorphin peptide levels and KOP-r signaling in the CeA of alcohol-dependent Wistar rats (induced by chronic intermittent alcohol vapor exposure) [Kissler et al., 2014]. This
enhanced KOP-r/dynorphin activity in the CeA may present a homeostatic adaptation of the CNS after chronic alcohol consumption or in negative affective state during alcohol withdrawal. Further work has found that KOP-r activation inhibits both GABAergic synaptic responses and alcohol effects in the CeA (and BNST), and regulates GABA release [Li et al., 2012; Park et al., 2013]. In the NAc shell, KOP-r blockade also reduces alcohol self-administration in alcohol-dependent rats [Nealey et al., 2011]. On the basis of the above evidence, it confirms that increased levels of KOP-r/dynorphin in the CeA and NAc play functional roles in regulation of negative affective state and/or reward after alcohol exposure or withdrawal [Shippenberg et al., 2007; Wee & Koob, 2010].

Though early work has found that “classic” KOP-r agonists attenuate alcohol drinking and alcohol CPP [Lindholm et al., 2001; Logrip et al., 2009], most “classic” KOP-r agonists produce sedation and dysphoria, and those side effects limit their potential for clinical use [e.g., Morani et al., 2009]. Therefore, the development of new KOP-r agonists with reduced side effects may have the potential to produce useful compounds for the treatment of alcoholism. Recently, there is rapidly growing research into the identification of functionally selective (biased) KOP-r full agonists or partial agonists for the development of anti-addictive compounds [Maillet et al., 2015; Simonson et al., 2015; White et al., 2015; Brust et al., 2016; Schattauer et al., 2017; Townsend et al., 2017; Zhou et al., 2017a]. For a good example, Mesyl Salvinorin B (MSB), an analogue of salvinorin A, is a potent KOP-r full agonist with fewer side effects (sedation and dysphoria) compared to other “classic” KOP-r agonists [Simonson et al., 2015; Zhou et al., 2017a]. We have further examined the pharmacological effects of MSB on excessive or “relapse” drinking in mice, to determine its potential for development as an anti-relapse compound for alcoholism. Acute administration of MSB significantly reduces both excessive drinking in an IA model and “relapse” drinking in a mouse ADE model in a dose-dependent manner [Zhou et al., 2017a, 2017d]. Nalfurafine (clinically available G-biased KOP-r agonist) with few side effects [Schattauer et al., 2017], also decreases excessive alcohol
drinking in mice [Zhou et al., 2018b]. These findings have shown promising in vivo results indicating that biased KOP-r full agonists may offer novel approaches to treat alcoholism, without traditional dysphoric properties of classic KOP-r agonists.

There are many studies that have demonstrated that classic KOP-r agonists increase alcohol drinking [Rose et al., 2016], and induce alcohol-seeking behavior in a reinstatement model [Funk et al., 2014] and “relapse” drinking in a ADE model [Hölter et al., 2000] (see an update review by Anderson and Becker in 2017). Therefore, our new data that the KOP-r full agonist MSB reduces, rather than triggers, relapse-like drinking, presents a scenario that seems opposite to the results using classic KOP-r agonists. After chronic excessive alcohol consumption, however, the endogenous dynorphin (a G-protein and beta-arrestin dependent agonist [Maillet et al., 2015; White et al., 2015]) and KOP-r systems are activated in several neuronal structures. Either the increased release of dynorphin [Marinelli et al., 2006] or enhanced KOP-r activity [Rose et al., 2016] produces sedation, dysphoria, anxiety- and depression-like behaviors that may drive excessive and “relapse” drinking [Tunstall et al., 2017]. In support of this concept, the dynorphin levels and KOP-r activity are found to be increased in the rat CeA after chronic alcohol exposure [D’Addario et al., 2013; Zhou et al., 2013c; Kissler et al., 2014]. Indeed, preclinical studies have demonstrated that the activation of p38 MAPK to stress-mediated dynorphin/KOP-r stimulation is linked to the beta-arrestin mediated transduction pathway [Bruchas et al., 2006, 2010]. Unlike dynorphin, however, MSB does not induce sedation or anhedonia in rats or mice [Simonson et al., 2015; Zhou et al., 2017a], and could act as a G-protein dependent (biased) agonist, which was suggested by our recent report [Simonson et al., 2015]. Nalfurafine, acting as a biased KOP-r agonist, could possibly compete with excessive dynorphin to bind the KOP-r, thereby reducing beta-arrestin signaling. This could be responsible, at least in part, for reducing excessive alcohol intake, as nalfurafine reverse the dynorphin-enhanced dysphoria, anxiety- or depression-like behavior during alcohol withdrawal. Together, these support the notion that biased KOP-r agonists exhibit different molecular,
cellular and behavioral properties than classic KOP-r agonists [Che et al., 2018]. Our study is in line with growing research into the development of biased KOP-r ligands for anti-addictive compounds [Maillet et al., 2015; White et al., 2015; Brust et al., 2016; Townsend et al., 2017].

**Conclusion and future directions**

As presented in this mini-review, there has been substantial progress in our understanding how alcohol exposure disrupts the CNS stress responsive systems to modulate alcohol-taking and seeking behaviors in several selective animal models. It has been well known that the MOP-r/POMC and KOP-r/dynorphin (endogenous opioid systems) clearly play critical roles in alcohol addiction, and specific alterations of their expression levels or receptor activity may affect stress responsivity and then contribute to vulnerability to develop the alcohol dependency or relapse. Other stress responsive systems discussed here (including the V1b receptor with AVP, FAAH with eCBs) are also potentially involved in alcohol addiction, as new evidence emerges in recent studies.

Combination medications targeting multiple neurotransmitter pathways may have increased efficacy over the traditional single-medication strategy. Actually, as discussed above, pharmacological and neurobiological studies have provided strong supporting findings, given that many stress responsive systems, including CRF, Neuropeptide Y, and glucocorticoid receptor, are profoundly disrupted after chronic alcohol exposure. Further studies on combination medications are needed to develop more effective new pharmacotherapies for treating alcoholism. Although NTN is more effective in people with alcoholism who have MOP-r variant A118G [Bond et al., 1998; Bart et al., 2004; Kreek and LaForge, 2007; Anton et al., 2008], the single-target pharmacotherapy has relatively modest therapeutic value, also suggesting a need for better efficacy [Müller et al., 2014]. By targeting multiple neurotransmitters implicated in different components of alcohol addiction, combination medications are expected to have greater efficacy than the single-medication therapy [Karoly et
al., 2015; Zhou and Leri, 2016]. There are several precedents to study the combinations of NTN with other compounds in rodent models, like acamprosate [Heyser et al., 2003], or prazosin [Froehlich et al., 2013]. Consistently, our recent studies in mouse alcohol escalation drinking models suggest that the combination of KOP-r agonist MSB, V1b antagonist SSR149415 or bupropion with NTN may be more efficacious in treating alcoholism than NTN alone [Zhou et al., 2017a, 2018a]: (1) the effects of these combined, low-dose administrations of MSB/NTN, SSR149415/NTN or bupropion/NTN on alcohol drinking are greater than those of either drug alone; and (2) the combinations show persistent effects after repeated administrations. In support of this notion, the effective medication nalmefene is a MOP-r antagonist plus a KOP-r partial agonist, targeting both MOP-r and KOP-r pathways and possibly synergistically reducing alcohol consumption. Indeed, most drugs (topiramate, varenicline and gabapentin) tested for alcoholism treatment target on multiple systems [Karoly et al., 2015]. Multiple targeting may have advantage for treating alcoholism as a multigenic disease. Therefore, we propose that the combination drugs may prove more effective than drugs that are highly selective for a single target.

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**Authorship contributions:** Wrote the manuscript [Y. Zhou]; contributed to the writing of the manuscript [M.J. Kreek].
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Young WS, Li J, Wersinger SR, and Palkovits M (2006) The vasopressin 1b receptor is prominent in the hippocampal area CA2 where it is unaffected by restraint stress or adrenalectomy. *Neuroscience* **143**: 1031-1039.


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Table 1. Effects of AVP or V1b antagonists on alcohol-related behaviors

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Sex</th>
<th>Model</th>
<th>Effect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brattleboro homozygote rats</td>
<td>male</td>
<td>2-bottle choice (24 h, 2-10%)</td>
<td>Decrease (intake) by systemic (osmotic pump) DGAVP</td>
<td>Rigter &amp; Crabbe (1985)</td>
</tr>
<tr>
<td>Rhesus monkeys</td>
<td>male</td>
<td>multiple-bottle choice (24 h, 1-8%)</td>
<td>Decrease (intake) by systemic (iv) DGAVP</td>
<td>Kornet et al. (1991)</td>
</tr>
<tr>
<td>Sardinian alcohol-preferring rats</td>
<td>male</td>
<td>2-bottle choice (24 h, 10%)</td>
<td>Decrease (intake and preference) by systemic (ip) SSR149415</td>
<td>Zhou et al. (2011a)</td>
</tr>
<tr>
<td>Alcohol-dependent Wistar rats</td>
<td>male</td>
<td>operant self-administration (10%, 30 min)</td>
<td>Decrease (intake) by systemic (ip) or intra-central amygdala SSR149415</td>
<td>Edwards et al. (2012)</td>
</tr>
<tr>
<td>C57Bl/6J mice</td>
<td>male</td>
<td>2-bottle choice (24 h, every other day, 15%)</td>
<td>Decrease (intake and preference) by systemic (ip) SSR149415</td>
<td>Zhou et al. (2018)</td>
</tr>
<tr>
<td>Human</td>
<td>male</td>
<td>phase II, double-blind, placebo-controlled randomized trial</td>
<td>Decrease (intake and relapse) by systemic (oral) ABT-436</td>
<td>Ryan et al. (2017)</td>
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<tr>
<td></td>
<td>female</td>
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</table>

ABT-436, V1b antagonist; DGAVP, desglycinamide-(Arg⁸)-vasopressin; SSR149415, V1b antagonist
Table 2  Effects of genetic deletion of β-endorphin, POMC and MOP-r on alcohol-related behaviors

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Sex</th>
<th>Model</th>
<th>Effect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-endorphin KO</td>
<td>male</td>
<td>2-bottle choice (24 h, 16%)</td>
<td>Decrease (intake and preference) with sex difference</td>
<td>Racz et al. (2008)</td>
</tr>
<tr>
<td>Tpit KO mice with pituitary-specific POMC deletion</td>
<td>male + female</td>
<td>1-bottle (4 h, 15%) in DID</td>
<td>No difference in intake or preference in either sex</td>
<td>Zhou et al. (2017b)</td>
</tr>
<tr>
<td>nPE KO mice with hypothalamic-specific POMC deletion</td>
<td>male + female</td>
<td>[1] 1-bottle (4 h, 7.5-30%) in DID; [2] 2-bottle choice (24 h, every other day, 7.5-30%) in IA; [3] ADE</td>
<td>Decrease (intake and preference) in all 3 models, with sex difference</td>
<td>Zhou et al. (2017b)</td>
</tr>
</tbody>
</table>

ADE, alcohol deprivation effect; CPP, conditioned place preference; DID, Drinking-in-the-dark; IA, intermittent access; KO, knockout; MOP-r, mu-opioid receptor; nPE, neuronal Pomc enhancers.
Table 3 Effects of genetic mutation or deletion of FAAH gene and of FAAH inhibitors on alcohol-related behaviors

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Sex</th>
<th>Model</th>
<th>Effect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAAH KO</td>
<td>male</td>
<td>2-bottle choice (24 h, 12-20%)</td>
<td>Increase (intake and preference), with sex difference</td>
<td>Basavarajappa et al. 2006</td>
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<tr>
<td></td>
<td>female</td>
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<tr>
<td>FAAH KO</td>
<td>male</td>
<td>[1] 2-bottle choice (24 h, 3-15%);</td>
<td>[1] Increase (intake and preference, with sex difference</td>
<td>Blednov et al. 2007</td>
</tr>
<tr>
<td>FAAH+/+ mice</td>
<td>male</td>
<td>2-bottle choice (24 h, 3-12%)</td>
<td>Increase (intake and preference by systemic (ip) URB597, with sex difference</td>
<td>Blednov et al. 2007</td>
</tr>
<tr>
<td></td>
<td>female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C57Bl/6J mice</td>
<td>male</td>
<td>[1] 1-bottle (4 h, 15%) in DID for 3 weeks;</td>
<td>Decrease (intake and preference) by systemic (ip) URB597, in IA and ADE (but not the DID) models, with no sex difference</td>
<td>Zhou et al. 2017c</td>
</tr>
<tr>
<td></td>
<td>female</td>
<td>[2] 2-bottle choice (24 h, every other day, 7.5-30%) in IA;</td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td>[3] ADE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marchigian Sardinian</td>
<td>male</td>
<td>operant self-administration (10%, 30 min)</td>
<td>Decrease (intake) by intra-central and basolateral amygdala URB597</td>
<td>Stopponi et al. 2018</td>
</tr>
<tr>
<td>alcohol-preferring rats</td>
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<tr>
<td>FAAH C385A Knock-in mice</td>
<td>male</td>
<td>1-bottle (4 h, 15%) in DID for 4 days and 2-bottle choice (4 h, 15% vs. water) on day 5</td>
<td>Increase (intake and preference)</td>
<td>Zhou et al. 2016</td>
</tr>
<tr>
<td></td>
<td>female</td>
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<tr>
<td>Human FAAH C385A SNP</td>
<td>male</td>
<td>2119 patients</td>
<td>Association with street drug use and problem drug/alcohol use</td>
<td>Sipe et al. 2002</td>
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<tr>
<td>Human FAAH C385A SNP</td>
<td>male</td>
<td>1434 European Americans with AD diagnosis</td>
<td>Association with probability and severity of alcohol dependence</td>
<td>Sloan et al. 2018</td>
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<tr>
<td></td>
<td>female</td>
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</table>

AD, alcohol dependence; ADE, alcohol deprivation effect; CPP, conditioned place preference; DID, Drinking-in-the-dark; FAAH, fatty acid amide hydrolase; IA, intermittent access; KO, knockout; SNP, single nucleotide polymorphisms; URB597, FAAH inhibitor
Table 4 Effects of KOP-r agonists or antagonists on alcohol drinking behaviors

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Sex</th>
<th>Model</th>
<th>Effect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wistar rats</td>
<td>male</td>
<td>4-bottle choice (24 h, 5-20%)</td>
<td>Increase (intake and preference) by systemic (minipump) enadoline No change by systemic (ip) nor-BNI</td>
<td>Holter et al. 2000</td>
</tr>
<tr>
<td>Lewis rats</td>
<td>male</td>
<td>2-bottle choice (2 h, 10%)</td>
<td>Decrease (intake) by systemic (ip) U50,488</td>
<td>Lindholm et al. 2001</td>
</tr>
<tr>
<td>Lewis rats</td>
<td>male</td>
<td>2-bottle choice (24 h, 10%)</td>
<td>Increase (intake) by systemic (sc) nor-BNI</td>
<td>Mitchell et al. 2005</td>
</tr>
<tr>
<td>C57Bl/6J mice</td>
<td>male</td>
<td>2-bottle choice (24 h, 3-10%)</td>
<td>Increase (intake) by systemic (ip) U50,488 Decrease (intake) by systemic (ip) nor-BNI in stressed mice</td>
<td>Sperling et al. 2010</td>
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<tr>
<td>C57Bl/6J mice</td>
<td>male</td>
<td>2-bottle choice (2 h, 10-15%)</td>
<td>Increase (intake and preference) by systemic (ip) U50,488</td>
<td>Rose et al. 2016</td>
</tr>
<tr>
<td>C57Bl/6J mice</td>
<td>male</td>
<td>1-bottle (1 h, 15%)</td>
<td>Increase (intake) by systemic (ip) U50,488 Decrease (intake) by systemic (ip) LY2444296 in stressed mice</td>
<td>Anderson et al. 2016</td>
</tr>
<tr>
<td>C57Bl/6J mice</td>
<td>male</td>
<td>[1] 1-bottle (4 h, 15%) in DID for 3 weeks; [2] 2-bottle choice (24 h, every other day, 7.5-30%) in IA</td>
<td>Decrease (intake and preference) by systemic (ip) MSB, in IA (but not the DID) model, with no sex difference Decrease (intake) by systemic (ip) nor-BNI in IA model, with sex difference</td>
<td>Zhou et al. 2017a</td>
</tr>
</tbody>
</table>

DID, Drinking-in-the-dark; IA, intermittent access; MSB, Mesyl Sal B (kappa agonist); norBNI, KOP-r antagonist; U50,488, KOP-r agonist