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Role of the Dynorphin/Kappa Opioid Receptor System in the Motivational Effects of Ethanol

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Abstract

Evidence has demonstrated that dynorphin (DYN) and the kappa opioid receptor (KOR) system contribute to various psychiatric disorders, including anxiety, depression, and addiction. More recently, this endogenous opioid system has received increased attention as a potential therapeutic target for treating alcohol use disorders. In this review, we provide an overview and synthesis of preclinical studies examining the influence of alcohol (ethanol) exposure on DYN/KOR expression and function, as well as studies examining the effects of DYN/KOR manipulation on ethanol's rewarding and aversive properties. We then describe work that has characterized effects of KOR activation and blockade on ethanol self-administration and ethanol dependence/withdrawal-related behaviors. Finally, we address how the DYN/KOR system may contribute to stress-ethanol interactions. Despite an apparent role for the DYN/KOR system in motivational effects of ethanol, support comes from relatively few studies. Nevertheless, review of this literature reveals several common themes: (1) rodent strains genetically predisposed to consume more ethanol generally appear to have reduced DYN/KOR tone in brain reward circuitry; (2) acute and chronic ethanol exposure typically upregulate the DYN/KOR system; (3) KOR antagonists reduce behavioral indices of negative affect associated with stress and chronic ethanol exposure/withdrawal; and (4) KOR antagonists are effective in reducing ethanol consumption, but are often more efficacious under conditions that engender high levels of consumption, such as dependence or stress exposure. These results support the contention that the DYN/KOR system plays a significant role in contributing to dependence- and stress-induced elevation in ethanol consumption. Overall, more comprehensive analyses (on both behavioral and mechanistic levels) are needed to provide additional insight into how the DYN/KOR system is engaged and adapts to influence the motivation effects of ethanol. This information will be critical for the development of new pharmacological agents targeting KORs as promising novel therapeutics for alcohol use disorders and comorbid affective disorders.

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Keywords

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Introduction

The opioid peptide family consists principally of endorphins, enkephalins, and dynorphins, which serve as endogenous ligands for mu, delta, and kappa opioid receptors. Dynorphins, which bind with greatest affinity to kappa opioid receptors, are derived from the precursor protein prodynorphin, which can be cleaved to form numerous active peptides including Dynorphin A (DYN-A₁₋₈, DYN-A₁₋₁₃, and DYN-A₁₋₁₇), Dynorphin B (DYN-B), Big Dynorphin (a 32-amino acid peptide comprised of both DYN-A and DYN-B), and α - and β -neoendorphins (Chavkin, 2013; Schwarzer, 2009). Kappa opioid receptors (KORs) are G protein-coupled receptors with seven transmembrane domains that, when activated, inhibit adenylyl cyclase and act through a variety of signaling cascades (Bruchas and Chavkin, 2010).

Dynorphin (DYN) and KORs are widely distributed throughout the brain. In human brain tissue, high levels of prodynorphin mRNA have been observed in striatal (nucleus accumbens, caudate nucleus, putamen) and limbic (particularly the amygdala, entorhinal cortex, and dentate gyrus) regions (Hurd, 1996). An assessment of human KOR distribution in the brain revealed high expression of transcript throughout the cortex, amygdala, striatum (nucleus accumbens, caudate, putamen), thalamus, hypothalamus, and olfactory bulb (Simonin et al., 1995).

In situ hybridization analysis of the C57BL/6J mouse also revealed intense prodynorphin mRNA expression in several brain regions including the olfactory tubercle, caudate putamen, central nucleus of the amygdala, paraventricular nucleus of the hypothalamus, and the nucleus of the solitary tract (Lin et al., 2006). Similarly, in situ hybridization analysis of KOR mRNA showed high expression in the claustrum, endopiriform nucleus, thalamus (paraventricular and parafascicular nuclei), hypothalamus (periventricular, preoptic, and ventromedial nuclei), substantia nigra pars compacta, and ventral tegmental area in mice. (DePaoli et al., 1994). A study examining KOR binding density and KOR mRNA expression in the rat brain reported overlap in many brain regions, including the olfactory tubercle, nucleus accumbens, bed nucleus of the stria terminalis, caudate putamen, amygdala, paraventricular nucleus of the hypothalamus, locus coeruleus, and nucleus of the solitary tract (Mansour et al., 1995). As the anatomical distribution of DYN and KOR expression extensively overlays with reward and stress pathways, the endogenous opioid system is well-positioned to influence affective states by altering stress- and reward-related signaling in the brain (Bruchas et al., 2010; Crowley and Kash, 2015; Wee and Koob, 2010).

Behavioral effects of KOR activation typically oppose effects observed with other opioid receptors. Whereas activation of mu opioid receptors is highly reinforcing, resulting in conditioned place preference and increased dopamine release in the nucleus accumbens, KOR activation produces aversive or dysphoric states, resulting in conditioned place aversion and reduced dopamine release in the nucleus accumbens (Mucha and Herz, 1985;

Di Chiara and Imperato, 1988). KOR agonists have also been shown to increase reward thresholds in intracranial self-stimulation procedures, suggesting an anhedonic effect (decreased sensitivity to rewarding stimuli) (Todtenkopf et al., 2004). In addition to inducing negative affective states, KOR activation also influences locomotor, feeding, and sexual behavior (Bruijnzeel, 2009).

There is a large body of evidence indicating that DYN and KOR activity contribute to various psychiatric disorders, including anxiety, depression, and addiction (Hang et al., 2015; Knoll et al., 2010; Wee and Koob, 2010; Butelman et al., 2012; Crowley and Kash, 2015; Lalanne et al., 2014). Dynamic neuroadaptive changes associated with development of alcohol (ethanol) dependence are suggested to involve recruitment of various stress-related neuropeptides, including the DYN/KOR system. These adaptations, in turn, are suggested to contribute to negative affective states that perpetuate excessive, unhealthy drinking (Koob, 2008). In recent years, the DYN/KOR system has received increased attention as a potential therapeutic target for the treatment of ethanol use disorders (Walker et al., 2012).

In this review, we provide an overview and synthesis of studies that have examined the influence of ethanol exposure on DYN/KOR expression and function, as well as studies focused on examining the effects of DYN/KOR manipulation on behavioral effects of ethanol. Given its role in mediating dysphoric/aversive states, a growing literature has implicated the DYN/KOR system in the motivational effects of ethanol. However, in many cases, the outcomes of these studies have been inconsistent. Here we focus on multiple areas of preclinical ethanol research, highlighting factors that may contribute to discrepant findings. First, we describe the influence of genetic factors and ethanol exposure on DYN/KOR expression in the brain. Second, we synthesize literature that has examined effects of KOR activation and blockade on ethanol-conditioned behaviors, home-cage ethanol consumption, and operant ethanol self-administration/reinstatement procedures. After presenting corresponding evidence from transgenic mice targeting the DYN/KOR system, we then discuss effects of KOR manipulation within the context of ethanol dependence and withdrawal-related behaviors. Finally, we describe the evidence for DYN/KOR system involvement in interactions of stress and ethanol reward and consumption.

Influence of Genetic Factors and Ethanol Exposure on DYN/KOR Expression

Basal Differences in DYN/KOR Expression

Reports of differential DYN/KOR expression within brain reward circuitry in rodent strains that differ in preference for ethanol suggest that genetic influences may modulate the effects of DYN/KOR activity on ethanol-related behaviors. In general, rodent strains genetically predisposed to consume more ethanol appear to have reduced DYN/KOR tone relative to strains that consume less ethanol. For example, reduced DYN in the ventral tegmental area and nucleus accumbens was reported in rats selectively bred to be alcohol-preferring (AA rats) relative to their non-preferring counterparts (ANA rats) (Nylander et al., 1994; Terenius, 1994). Similarly, Lewis rats, an inbred strain that readily self-administers ethanol

and other drugs of abuse, exhibit less DYN-A in the ventral tegmental area compared to the Fischer 344 inbred rat strain (Nylander et al., 1995). Ethanol-avoiding DBA/2 mice were reported to show greater DYN-A₁₋₁₃ and DYN-A₁₋₈ expression in the nucleus accumbens and caudate putamen than ethanol-preferring C57BL/6 mice (Jamensky and Gianoulakis, 1997). Similarly, DBA/2 mice also exhibited greater DYN-B in nucleus accumbens and striatum relative to C57BL/6 mice (Ploj et al., 2000). However, C57BL/6 mice were reported to have higher KOR binding sites and DYN-A₁₋₁₃ levels in the amygdala and more DYN-A₁₋₈ in the ventral tegmental area than DBA/2 mice (Jamensky and Gianoulakis, 1997). Thus, differences in DYN/KOR expression between inbred mouse strains may depend on brain region examined as well as differences in peptide vs. receptor measurements.

Effects of Ethanol Exposure on DYN/KOR Expression and Activity

Both self-administered and experimenter-administered acute and chronic ethanol exposure typically result in an upregulation of the DYN/KOR system. Studies using in vivo microdialysis procedures have shown that acute systemic administration of ethanol produces dose-related increases in DYN-A₁₋₈ levels in the nucleus accumbens (Marinelli et al., 2006), central nucleus of the amygdala (Lam et al., 2008), and ventral tegmental area (Jarjour et al., 2009). One day of intragastric binge-like ethanol exposure (4.5 g/kg total) was sufficient to elevate prodynorphin mRNA expression in both the amygdala and prefrontal cortex of adult Sprague-Dawley rats (D'Addario et al., 2013). Six days of intragastric ethanol exposure (4.3 g/kg/day) resulted in elevated DYN-A and DYN-B in the hippocampus of Wistar rats 3 or 7 days after the final exposure (Kuzmin et al., 2013). Acute injection (1 g/kg) or one month of voluntary ethanol consumption both resulted in elevated DYN mRNA and peptide levels in the paraventricular nucleus of the hypothalamus in rats (Chang et al., 2007). Elevated prodynorphin levels were reported in the nucleus accumbens of rats with a 1-month history of drinking ethanol (Przewlocka et al., 1997). Similarly, in rats injected with ethanol (2 g/kg) for 13 days, DYN-B was upregulated in the nucleus accumbens 30 minutes later and 21 days later (Lindholm et al., 2000). In contrast to these reports of elevated expression of the opioid peptide, repeated ethanol (2 g/kg) injections over 14 days reduced KOR mRNA expression in the nucleus accumbens and ventral tegmental area (Rosin et al., 1999).

Adaptations in the DYN/KOR system have been also observed during ethanol withdrawal. For example, increased DYN-A stimulated GTP γ S coupling and immunoreactivity were reported in the amygdala during withdrawal from chronic ethanol vapor exposure, indicative of greater KOR signaling and dynorphin peptide expression (Kissler et al., 2014). The DYN/KOR system also has been implicated in ethanol withdrawal-related seizures. Using mice selectively bred for high and low seizure susceptibility during ethanol withdrawal, greater prodynorphin mRNA, greater KOR binding, and greater KOR density were observed in withdrawal seizure-prone (WSP) mice relative to withdrawal seizure-resistant (WSR) mice following withdrawal from chronic ethanol vapor exposure (Beadles-Bohling et al., 2000; Beadles-Bohling and Wiren, 2005).

Taken together, a modest literature suggests that acute and chronic ethanol exposure and withdrawal produce changes in DYN/KOR expression and activity. However, these changes appear dependent on a host of biological and experimental variables, including genetic

factors, magnitude of ethanol dose/exposure, and brain region examined. Effects of ethanol on the DYN/KOR system may also depend on whether expression of peptides or receptors is measured. Clearly, there is a need for additional studies that systematically address these variables to better elucidate conditions and mechanisms by which ethanol alters expression and function of the DYN/KOR system. Linking such changes in specific brain regions with ethanol-relevant behavioral phenotypes should be a primary goal of this work.

Effects of DYN/KOR Activity on Ethanol-Conditioned Behavior and Consumption

Ethanol Conditioned Reward and Aversion

Several studies have examined the behavioral effects of pharmacological manipulation of KORs using models of ethanol-conditioned reward and aversion. In these models, experimenter-administered ethanol is given either before confinement to one side of a multi-compartment chamber (for assessment of conditioned place preference; see Cunningham et al., 2006) or after consumption of a novel tastant (for assessment of conditioned taste avoidance; see Davis and Riley, 2010). As shown in Table 1, results from these studies have been somewhat inconsistent, with numerous experimental variables likely contributing to the discrepant findings.

Activation of KORs with the agonist U50,488 has been reported to both block and potentiate ethanol-induced conditioned place preference (CPP). While these studies have employed different rodent species and strains, it appears that dose and timing of U50,488 administration may be the most critical factors in determining the direction of study outcomes. In two studies in which U50,488 (0.3 – 3 mg/kg) was administered shortly (i.e., 0 – 10 min) before ethanol injections during the conditioning phase, subjects spent less time on the ethanol-paired side during the CPP test session (Logrip et al., 2009; Matsuzawa et al., 1999). That is, under these experimental conditions, the KOR agonist attenuated ethanol-induced CPP. In contrast, opposite results were observed in another study that examined a higher dose of U50,488 (10 mg/kg) with a longer pretreatment time (90 min before ethanol), suggesting that under these conditions, KOR activation potentiated expression of ethanol-induced CPP (Sperling et al., 2010). At present, it is unclear how dose or timing (or both variables) of a KOR agonist interacts with ethanol to produce opposite effects on the conditioned rewarding effects of ethanol. Additional studies focused on underlying mechanisms may help clarify this issue.

A few studies have examined the effects of KOR antagonists on ethanol-induced CPP or CTA. One study in female mice reported similar ethanol-induced CPP in subjects pretreated with the long-acting KOR antagonist nor-binaltorphimine (nor-BNI) relative to saline, but when subjects were retested in the presence of ethanol (referred to as state-dependent CPP), a greater CPP effect was observed in nor-BNI treated mice (Nguyen et al., 2012). Another study reported no effects of nor-BNI treatment on ethanol CPP in non-stressed male mice (Sperling et al., 2010). Two studies conducted in rats have shown that nor-BNI does not alter ethanol-induced CTA whether administered 24 hr before conditioning (Roma et al., 2008) or 24 hr before testing (Anderson et al., 2013). Collectively, these findings suggest that KOR

blockade does not influence ethanol-induced CTA or CPP (at least in standard paradigms that assess these effects in an ethanol-free state). Studies focused on stressed-induced ethanol CPP are described later in this review.

In sum, given the role of the DYN/KOR system in mediating negative affect and aversive-related behaviors (Al-Hasani et al., 2015; Land et al., 2008; Van't Veer and Carlezon, 2013), it is not surprising that KOR activation has been shown to alter ethanol-conditioned reward. However, there is a paucity of studies devoted to this issue and, as outlined in Table 1, results from these studies have been generally inconsistent. Dose and timing of agonists in relation to ethanol administration appear to be critical factors in influencing effects of KOR activation on ethanol-induced CPP. Additional work in this area will be needed to better define how modulation of KOR activity influences ethanol-related conditioned reward and aversion.

Home-cage Ethanol Drinking

A number of studies have assessed effects of pharmacological activation or blockade of KORs on ethanol consumption using models of home-cage drinking (Table 2). Most of these studies involved free-choice drinking using a 2-bottle paradigm (ethanol vs. water), with ethanol consumption measured under either continuous or limited access conditions.

The majority of studies investigating the effects of KOR activation in various rat strains report a decrease in ethanol consumption following systemic or intra-hypothalamic administration of the KOR agonist U50,488. Interestingly, all of the studies conducted in C57BL/6J mice involving KOR agonists report increased ethanol consumption, although one study reported this increase only in mice subjected to social stress (Kudryavtseva et al., 2006). Thus, there is an apparent species difference with regard to effects of a KOR agonist on ethanol consumption in a 2-bottle choice situation. Interestingly, differences in timing of drug administration or duration of access to ethanol do not appear to explain the apparent opposite effects reported in rats and mice.

Reports of KOR blockade on home-cage ethanol consumption are more consistent, with most studies demonstrating reduced intake (Table 2). However, numerous reports observed that antagonism of KORs reduced ethanol intake in stressed or ethanol-dependent subjects, but not in non-stressed or non-dependent subjects (Karkhanis et al., 2016a; Sperling et al., 2010; Anderson et al., 2016; Rose et al., 2016). These findings are described in greater detail later in this review. Although minimal work has examined sex differences, a couple of reports have suggested that effects of KOR antagonism on home-cage drinking may differ between male and female subjects. Specifically, nor-BNI was reported to increase ethanol consumption in male rats while decreasing ethanol intake in female rats (Morales et al., 2014). Conversely, a recent study reported that nor-BNI administration reduced ethanol consumption in male mice, but had no effect in female mice (Zhou et al., 2017).

Operant Ethanol Self-Administration and Relapse-Like Behavior

As outlined in Table 3, a number of studies have employed operant conditioning procedures to examine the effects of manipulating KOR activity on ethanol responding and consumption. All of this work has been conducted in rats. KOR activation was reported to

decrease operant oral ethanol self-administration in selectively bred ethanol-preferring rats (P rats) as well as non-selected Long-Evans rats (Henderson-Redmond and Czachowski, 2014).

A greater number of studies have assessed consequences of KOR blockade on operant ethanol self-administration. As in home-cage drinking studies, KOR antagonists typically reduced self-administration (e.g., Schank et al., 2012a; Cashman and Azar, 2014; Rorick-Kehn et al., 2014). Further, the ability of KOR blockade to reduce ethanol self-administration was consistently shown to be selective for subjects demonstrating elevated consumption associated with ethanol dependence (e.g., Kissler et al., 2014; Walker et al., 2011; Walker et al., 2008). In a study that observed reduced ethanol self-administration among non-dependent subjects following KOR antagonist administration (Schank et al., 2012a), twice the dose of nor-BNI (30 mg/kg) was required to produce an effect similar to that observed at a lower dose (15 mg/kg) in ethanol-dependent rats (Walker et al., 2011). This provides additional evidence that ethanol-dependent subjects are more sensitive to effects of KOR antagonists on drinking compared to non-dependent subjects. Non-dependent P-rats were also shown to be sensitive to the effects of nor-BNI on ethanol self-administration responding under fixed and progressive ratio schedules, spontaneous recovery, and relapse (Cashman and Azar, 2014; Rorick-Kehn et al., 2014; Deehan et al., 2012). To our knowledge, differences in DYN tone and/or KOR expression in P-rats compared to NP-rats or their progenitor strain (Wistar rats) have not been characterized, but may potentially contribute to differences in sensitivity of these rats to KOR antagonism.

Finally, cue-induced reinstatement of ethanol responding has been shown to be reduced by the KOR antagonists nor-BNI and JDTic (Berger et al., 2013; Schank et al., 2012a). Interestingly, although KOR antagonists have been shown to attenuate stress-induced reinstatement of responding for drugs of abuse such as cocaine (Graziane et al., 2013; Polter et al., 2014), this effect has not been observed with ethanol (see Schank et al., 2012a). Providing further evidence for a distinction between cue- and stress-induced reinstatement, naltrexone, a nonspecific opioid antagonist, was reported to block cue but not footshock stress-induced reinstatement of operant responding for ethanol (Le et al., 1999; Liu and Weiss, 2002). KOR antagonists, however, were shown to block reinstatement of ethanol seeking behavior induced by the pharmacological stressor yohimbine (Funk et al., 2014). Of note, two studies reported that systemic administration of the KOR agonist U50,488 reinstated responding for ethanol after extinction (Funk et al., 2014; Harshberger et al., 2016). These findings suggest that KOR activation may underlie, at least in part, the ability of stress to provoke relapse-like responding for ethanol. Further discussion of interactions of stress and ethanol occur later in this review.

Collectively, a growing body of literature indicates that KOR antagonists are effective in reducing operant responding for ethanol, and efficacy of these agents appears to be especially apparent under conditions that engender high levels of ethanol intake (drinking associated with dependence), perhaps driven by an upregulation of the DYN/KOR system.

Genetic Models of DYN/KOR Activity and Ethanol-Conditioned Behavior and Consumption

Ethanol Conditioned Reward and Aversion

Only three studies have employed genetic mouse models to examine the role of DYN/KOR activity in ethanol-conditioned reward and aversion (Table 4). In accordance with the pharmacological antagonism studies described above, male and female prodynorphin knockout mice were shown to express the same degree of ethanol-induced CPP as their wild-type counterparts when tested in an ethanol-free state (Blednov et al., 2006; Nguyen et al., 2012), whereas female prodynorphin knockout mice exhibited greater CPP relative to wildtype controls when tested in the presence of ethanol (i.e., state-dependent CPP) (Nguyen et al., 2012). Another study reported greater ethanol-induced CPP in male prodynorphin knockout mice (Femenia and Manzanares, 2012). However, this study involved ethanol administration via oral gavage immediately prior to conditioning, a procedural variation that may have contributed to the contrasting outcome. A single report of ethanol-induced CTA in prodynorphin knockout mice observed no changes relative to wild-type mice (Blednov et al., 2006). Thus, genetic deletion of the precursor for DYN generally produced results that are congruent with pharmacological blockade of KORs.

Home-cage Ethanol Drinking

Studies using genetic models have also reported mixed findings and equivocal evidence for sex differences in home-cage drinking (Table 4). For example, null mutant female, but not male, mice lacking prodynorphin showed reduced ethanol intake compared to wildtype littermates (Blednov et al., 2006). In another study, both male and female prodynorphin knockout mice consumed more ethanol than wildtype controls in a two-bottle choice home-cage drinking paradigm (Racz et al., 2013). A similar outcome was reported in male prodynorphin knockout mice (Femenia and Manzanares, 2012). There is also a report indicating male dynorphin-deficient mice consumed a similar amount of ethanol compared to their wildtype controls (Sperling et al., 2010). It is unclear whether differences in genotype among the prodynorphin knockout animals used in these studies can explain differences in the reported outcomes. Two studies using transgenic mice targeting the KOR have examined voluntary (2-bottle choice) ethanol consumption in the home-cage, with reduced ethanol intake reported in male (Kovacs et al., 2005) and female (Van't Veer et al., 2016) KOR knockout mice. Interestingly, binge-like ethanol consumption did not differ between KOR null mutant and wildtype female mice (Van't Veer et al., 2016).

Effects of DYN/KOR Activity on Ethanol Dependence/Withdrawal-Related Behaviors

Dependence/Withdrawal-Related Ethanol Consumption

As noted above, KOR antagonists have been reported to significantly reduce elevated ethanol consumption in ethanol dependent animals. In a series of studies, Walker and colleagues have demonstrated that the KOR antagonist nor-BNI reduced escalation of ethanol consumption observed in dependent rats without influencing consumption in non-

dependent animals (Walker and Koob, 2008; Walker et al., 2011, Kissler et al., 2014). Similar effects were reported in a recent study involving ethanol dependent mice (Rose et al., 2016). In another study that reported stress-induced enhancement of escalated drinking in dependent mice, the novel short-acting KOR antagonist, LY2444296 (Mitch et al., 2011), was shown to significantly attenuate this effect (Anderson et al., 2016). Taken together, there is converging evidence that pharmacological blockade of KORs reduces escalated drinking associated with dependence. These results appear congruent with previously noted evidence that ethanol dependence, particularly induced by chronic ethanol vapor inhalation, up-regulates the DYN/KOR system (e.g., Kissler et al., 2014).

Interestingly, effects of KOR agonists on ethanol consumption appear to be less selective. For example, the KOR agonist U50,488 was reported to produce a similar increase in ethanol consumption in both dependent and non-dependent mice (Anderson et al., 2016), suggesting similar sensitivity to direct KOR activation in increasing ethanol intake regardless of a history of ethanol exposure. In another study, systemic administration of U50,488 elevated ethanol consumption in non-dependent mice (Rose et al., 2016). Thus, in contrast to KOR antagonism selectively reducing ethanol consumption in dependent animals, these results suggest that systemic administration of a KOR agonist effectively increases ethanol drinking in both dependent and non-dependent subjects. Specific mechanisms underlying this effect (e.g., dependence-related adaptations in DYN synthesis/release and/or changes in KOR expression/function) have yet to be fully elucidated.

Dependence/Withdrawal-Related Behaviors

Pharmacological blockade of KORs has also been shown to reduce dysphoric-related behaviors associated with ethanol withdrawal. For example, nor-BNI attenuated withdrawal-induced anxiety-like behavior (indexed by time spent in the open arm of an elevated plus maze) in ethanol dependent rats, while having no effect on the same behavior in control (non-dependent) rats (Valdez and Harshberger, 2012). Similar results were reported in mice, with nor-BNI reducing marble-burying behavior during early withdrawal in ethanol dependent mice but not influencing this behavior in control mice (Rose et al., 2016). Another measure of negative affect associated with ethanol withdrawal (increased spontaneous 22-kHz ultrasonic vocalizations) was attenuated by nor-BNI administration in ethanol dependent rats, although these effects were not assessed in non-dependent rats (Berger et al., 2013). Another long-acting KOR antagonist, JD1c, was also shown to block anxiety-like behavior in the elevated plus-maze task during ethanol 'hangover' (i.e., acute withdrawal) in non-dependent rats (Schank et al., 2012a). Interestingly, the KOR agonist U50,488 produced anxiety-like behavior in the elevated plus-maze in both dependent and non-dependent rats, but ethanol dependent rats appeared more sensitive to the motor-impairing effects of the drug (Gillett, Harshberger, & Valdez, 2013). While these relatively few studies provide some evidence that DYN/KOR activation contributes to withdrawal-related dysphoria, there is a dearth of information regarding mechanisms and circuitry underlying these effects.

Finally, in addition to evidence for KOR involvement in dysphoria/negative affect related to ethanol withdrawal, two preclinical studies have examined effects of KOR antagonism on

physical symptoms of withdrawal following ethanol vapor exposure. In rats, nor-BNI had no effect on acute or protracted withdrawal symptoms including ventromedial distal limb flexion response, tail rigidity, and gait abnormalities (Kissler and Walker, 2016). However, another report found that nor-BNI increased withdrawal seizure severity (assessed by measuring handling-induced convulsions) in a seizure-resistant inbred mouse strain (Beadles-Bohling and Wiren, 2006). These findings were somewhat corroborated by another report that compared handling-induced convulsions during withdrawal from acute ethanol (4 g/kg, po) in prodynorphin knockout and wild-type mice (Femenia and Manzanares, 2012). Additional research is needed to further characterize these effects in preclinical models, especially if KOR antagonists are to be considered as potential therapeutics for alcohol use disorder.

Effects of DYN/KOR Activity on Stress-Ethanol Interactions

Although a large literature provides evidence that stress activates and upregulates the DYN/KOR system and supports a role for DYN/KOR activity in mediating behavioral responses to various stress events (Crowley and Kash, 2015; Knoll and Carlezon, 2010; Van't Veer and Carlezon, 2013), fewer studies have examined DYN/KOR system involvement in stress-ethanol interactions. In one report, nor-BNI blocked stress-enhanced ethanol CPP in mice exposed to forced swim stress prior to conditioning (Sperling et al., 2010). In contrast, nor-BNI further enhanced ethanol-induced CPP in rats exposed to a stress-associated context (fear) prior to the CPP procedure, an effect that was not examined in non-stressed subjects (Matsuzawa et al., 1999). In yet another account, nor-BNI blocked expression of ethanol-induced CTA in stressed rats, but not their non-stressed counterparts (Anderson et al., 2013). Thus, although stressed animals may be more sensitive to effects of KOR antagonism on ethanol reward, the direction of the reported effects has been inconsistent. Because relatively few studies have addressed this issue, it is difficult to identify experimental factors that might contribute to these discrepant results.

Effects of KOR activation and blockade on ethanol consumption also appear to be influenced by stress conditions. When mice were repeatedly subjected to social interactions in which they were identified as defeated or victorious, defeated mice consumed more ethanol and showed a further increase when treated with the KOR agonist U50,488 (Kudryavtseva et al., 2006). In another study, mice with a history of stress and ethanol dependence were more sensitive to the effects of KOR blockade on ethanol intake. That is, a short-acting KOR antagonist blocked stress-induced enhancement of ethanol consumption observed in dependent mice but did not alter intake in non-dependent mice (Anderson et al., 2016). These results generally align with studies involving use of transgenic mice. That is, whereas prodynorphin knockout mice consumed a similar amount of ethanol as their wildtype littermates (two-bottle choice drinking in the home-cage), the prodynorphin-deficient mice did not exhibit stress-enhanced drinking observed in wildtype mice (Sperling et al., 2010). In another study, prodynorphin-deficient male and female mice consumed more ethanol compared to their wildtype littermates in a home-cage free-choice drinking paradigm, but the null mutant mice did not exhibit a transient increase in drinking following footshock stress that was observed in the wildtype mice (Racz et al., 2013). Thus, the ability of stress to elevate ethanol consumption is lost when dynorphin is genetically deleted or

KORs are pharmacologically blocked. Collectively, these results suggest that the DYN/KOR system plays a role in contributing to stress-induced elevation in ethanol consumption.

Recent evidence suggests that early developmental stress experience can influence DYN/KOR system modulation of neurochemical and behavioral effects of ethanol. Jones and colleagues reported that social isolation during adolescence resulted in reduced basal dopamine in the nucleus accumbens and elevated ethanol consumption in adult rats, effects that were both blocked by the KOR antagonist nor-BNI (Karkhanis et al., 2016b). Further, rats with early stress (isolation housing) experience evidenced greater sensitivity to the ability of the KOR agonist U50,488 to reduce dopamine release in the nucleus accumbens in comparison to their group-housed counterparts. These results suggest that the DYN/KOR system is sensitized in animals subjected to early life stress and consequent elevated drinking may be mitigated by pharmacological blockade of KORs. Additional studies probing how the DYN/KOR system is influenced by stress and ethanol exposure, and how such changes may influence motivational effects of ethanol are certainly warranted.

Discussion

There is a growing body of literature implicating a role for DYN/KOR system activity in the motivational effects of ethanol. This review primarily focuses on evidence demonstrating that acute and chronic ethanol exposure alters DYN/KOR expression and function, and pharmacological manipulation of DYN/KOR activity influences ethanol reward as well as self-administration. As highlighted in this paper, results from this work have often produced conflicting outcomes. No doubt, the discrepant findings may be attributed to differences in numerous experimental parameters. Nevertheless, a few common themes emerge from this literature that point to critical variables that need to be considered in interpreting these findings as well as considerations for future work in this important, clinically relevant area of research.

Genetic Influences

Animal studies have provided some evidence for genetic factors influencing the role of the DYN/KOR system in motivational effects of ethanol. In some cases, clear species differences were noted (e.g., effects of KOR activation on home-cage ethanol drinking), but in most instances results in rats and mice were similar. Studies using inbred rodent strains and lines of rats/mice selectively bred for specific ethanol-related phenotypes have revealed differences in basal and ethanol-induced changes in DYN/KOR expression and function. Whether these differences contribute to related genetically-based behavioral differences is unclear. Given the rich information available on genetically-governed differences in ethanol drinking and related behaviors, expanded use of genetic models for studying DYN/KOR system involvement in these behaviors seems fruitful for future work. A limited number of studies have exploited use of animal models to examine how genetic-mediated deficiencies or overexpression of DYN/KOR may contribute to motivational effects of ethanol. Targeting such changes in specific brain regions will aid in elucidating mechanisms and circuitry underlying such effects.

Finally, while some human studies have revealed a potential role for KOR gene (OPRK1) polymorphism in risk for and/or treatment of ethanol dependence (Ashenhurst et al., 2012; Xuei et al., 2006; Zhang et al., 2008), other studies have produced contradictory findings (Arias et al., 2008; Gelernter et al., 2007). Variations in the prodynorphin gene have also been associated with negative craving for alcohol (i.e., seeking alcohol for relief from a negative emotional state) and alcohol dependence (Karpyak et al., 2013). DNA methylation (associated with reduced gene expression) in the prodynorphin gene was increased in alcoholics relative to control subjects in cortex and blood cells (Taqi et al., 2011; D'Addario et al., 2017). Future work using animal models may be valuable in clarifying whether allelic variants of OPRK1 or epigenetic factors (e.g., differential methylation of the prodynorphin gene) significantly contribute to risk for alcohol use disorder (AUD) and/or treatment responsiveness.

History of Ethanol Exposure

Synthesis of the literature reviewed in this paper indicates that a history of ethanol exposure plays an important role in modifying how KOR pharmacological agents influence motivational effects of ethanol. Much of this work has focused on effects of KOR agonists and antagonists on ethanol self-administration. Specifically, many studies have shown that KOR antagonists are especially effective in reducing elevated ethanol intake in dependent animals with little or no effect on more modest ethanol consumption in non-dependent animals (Kissler and Walker, 2016; Rose et al., 2016; Walker and Koob, 2008; Walker et al., 2012). This falls in line with the notion that while DYN/KOR activity may not play a significant role in initially regulating ethanol consumption, chronic ethanol exposure engages the DYN/KOR system in a manner that mediates and/or promotes increased motivation to drink (see Koob, 2013; Tejeda et al., 2012). Adaptations in DYN/KOR signaling following chronic ethanol exposure also have been shown to contribute to negative affect and dysphoria associated with withdrawal, effects that may fuel desire to engage in excessive levels of ethanol intake (Kissler et al., 2014; Kissler and Walker, 2016; Rose et al., 2016).

In contrast, KOR agonists are reported to similarly increase ethanol consumption in both dependent and non-dependent animals (Anderson et al., 2016). Thus, direct activation of KORs can mimic elevated ethanol consumption observed following chronic ethanol exposure. Interestingly, although KOR agonists appear to exhibit similar efficacy in increasing ethanol consumption in dependent and non-dependent animals, chronic ethanol exposure has been shown to enhance sensitivity to KOR-induced decreases in accumbal dopamine transmission (Karkhanis et al., 2016a; Rose et al., 2016). This upregulated KOR function in the nucleus accumbens (reducing dopamine tone) along with increased KOR function in the central amygdala are thought to underlie ethanol withdrawal-related behaviors reflective of dysphoria and, in turn, increased motivation to drink (Kissler et al., 2014; Rose et al., 2016). Further studies are needed to fully characterize how chronic ethanol exposure alters functional activity of the DYN/KOR system and how such adaptations relate to relevant behavioral effects.

Dose/Timing of DYN/KOR Pharmacological Manipulations

Dose and timing variables appear to be important factors that define how KOR pharmacological agents influence ethanol effects, particularly conditioned ethanol reward. For example, the KOR agonist U50,488 has been shown to both block and potentiate ethanol-induced CPP. In general, studies showing that U50,488 enhanced ethanol CPP used doses of the agonist that were shown in other studies to produce a conditioned place aversion when given alone (McLaughlin et al., 2006). Studies demonstrating reduced ethanol CPP used lower doses of U50,488 that did not produce a conditioned aversion effect alone (Logrip et al., 2009). In addition to U50,488 dose, the relative timing of KOR activation in relation to ethanol administration appears to have an important influence on these study outcomes. That is, studies using a short pretreatment time (0–10 minutes before conditioning) report that U50,488 blocks ethanol-induced CPP (Logrip et al., 2009; Matsuzawa et al., 1999), whereas studies with a longer pretreatment time (90 minutes) report potentiation of ethanol CPP (Sperling et al., 2010). Interestingly, U50,488 alone elicited a significant conditioned place aversion when administered 15 minutes prior to conditioning trials, with no aversion observed when the agonist was given 30 or 60 minutes prior to conditioning trials (McLaughlin et al., 2006). The importance of timing of U50,488 administration is also observed in studies involving the conditioned rewarding effects of other drugs. For example, multiple reports have shown that U50,488 given 60 minutes before cocaine administration potentiates CPP whereas a 15-minute pretreatment time results in place aversion (McLaughlin et al., 2006; Ehrich et al., 2014). Differential effects of timing of KOR agonist administration on cocaine-induced dopamine release in the nucleus accumbens have also been reported (Ehrich et al., 2014; Chartoff et al., 2016), an effect that has not yet been examined within the context of ethanol-induced dopamine release.

Conflicting accounts of the selectivity and time-dependent nature of KOR antagonist effects have also been reported. Two commonly used antagonists, nor-BNI and JDtic, both have a slow onset of action, with peak efficacy and selectivity often observed 24 hr after systemic administration (Carroll et al., 2004; Endoh et al., 1992; Broadbear et al., 1994). However, two studies that reported behavioral effects of nor-BNI or JDtic 2 hr but not 24 hr after administration contradict this assertion (Schank et al., 2012a; Funk et al., 2014). Further complicating interpretation of the pharmacological properties of nor-BNI, some older studies have reported mu opioid receptor antagonist properties up to 2 hours after administration (e.g., Endoh et al., 1992; Broadbear et al. 1994). Another report demonstrated dose- and time-dependent mu antagonist activity of nor-BNI, suggesting that higher doses of the KOR antagonist extend the time course of mu antagonist effects (Wettstein and Grouhel, 1996). Finally, a more recent report calls into question the selectivity of nor-BNI by demonstrating efficacy of the drug in mice lacking KOR receptors (Zhou et al., 2015). These characteristics suggest that, when reporting behavioral effects less than 24 hours after systemic administration of nor-BNI or JDtic, it may be important to rule out contributions of activity at mu receptors (see Schank et al., 2012a). Understanding the time course and magnitude of altered KOR activity/signaling produced by these pharmacological agents will be critical in the interpretation of behavioral results. Development of new, short-acting, and more selective KOR antagonists will be useful in clarifying this issue as well.

Expanded Analyses of Behaviors

In surveying the literature on contributions of the DYN/KOR system to motivational effects of ethanol, it is apparent that several models and experimental paradigms have been employed to broadly examine this issue. However, more comprehensive behavioral analysis would be helpful in better defining the role of this neuropeptide system in ethanol actions as well as clarifying discrepancies in the extant literature. For example, to our knowledge, no studies have examined how changes in DYN/KOR activity following chronic ethanol exposure may influence ethanol conditioned reward (or aversion). Most studies examining the role of DYN/KOR activity in ethanol withdrawal-related dysphoria have focused on anxiety and have utilized the elevated-plus maze procedure (e.g., Gillett et al., 2013; Schank et al., 2012a; Valdez et al., 2012). Expanding analysis to other behavioral indices of anxiety would further strengthen the purported link between upregulated DYN/KOR system and dysphoria/negative affect associated with ethanol withdrawal. Behavioral measures of anhedonia (e.g., brain stimulation reward model utilizing intracranial self-stimulation procedures) would be useful and also may provide opportunities to track the time course of such behavioral changes in relation to adaptations in DYN/KOR activity. Like ethanol exposure, stress is known to be a potent activator of the DYN/KOR system, yet relatively few studies have focused on how such changes may influence the motivational effects of ethanol. The complex reciprocal relationship between stress and ethanol is beginning to be more fully elucidated at the brain and behavior levels (Becker, 2012; Becker et al., 2011; Koob, 2013; Noori et al., 2014; Sinha, 2012; Spanagel et al., 2014; Uhart and Wand, 2009). Studies aimed at understanding the contribution of the DYN/KOR system to this dynamic ethanol-stress relationship are timely and needed in the field. This, in turn, will provide valuable insight about KOR pharmacological agents as potential therapeutics for comorbidity of alcohol use disorders and stress-related disorders (depression, PTSD) (Crowley and Kash, 2015; Lalanne et al., 2014).

Because the DYN/KOR system has been shown to play a role in the regulation of water balance and feeding behavior, it is important to establish selectivity of pharmacological effects for ethanol consumption. Indeed, studies conducted in transgenic mice suggest that genetic deletion of KORs or prodynorphin alters intake of saccharin and sucrose solutions (Kovacs et al., 2005; Blednov et al., 2006). Fortunately, many of the studies in this review included control solutions for comparison. For example, the KOR agonist U50,488 (at doses that reduced operant self-administration of 10% ethanol) also reduced self-administration of 2% sucrose (Henderson-Redmond and Czachowski, 2014). Another KOR agonist, Mesyl Salvinorin B, reduced ethanol intake but did not influence homecage consumption of 4% sucrose or 0.1% saccharin (Zhou et al., 2017). Effects of KOR antagonism are more consistent, with the majority of reports observing no effects of KOR antagonists on intake of sweetened control solutions (e.g., Nestby et al., 1999; Morales et al., 2014; Schank et al., 2012a; Doyon et al., 2006) or water (e.g., Lindholm et al., 2001; Mitchell et al., 2005; Rorick-Kehn et al., 2014) in homecage or operant self-administration procedures.

The DYN/KOR system has also been linked to ethanol-related cognitive impairment. One study conducted in Wistar rats found that six days of forced binge-like ethanol administration resulted in cognitive deficits (i.e., increased escape latency) in a water maze

task and elevated DYN levels in the hippocampus (Kuzmin et al., 2013). Treatment with nor-BNI (either systemic or in the dorsal CA3 region of the hippocampus) restored escape behavior in the ethanol-exposed rats whereas systemic nor-BNI did not enhance performance in control subjects (Kuzmin et al., 2013). In accordance with these preclinical findings, a comparison of postmortem human brain tissue in alcoholic and control subjects revealed greater expression of prodynorphin and KOR mRNA in areas implicated in the cognitive control of addictive behavior — dorsolateral PFC, orbitofrontal cortex, and hippocampus (Bazov et al., 2013). Clearly, this is an area of therapeutic opportunity in which more research is needed.

Finally, although a couple of studies have reported differential effects of nor-BNI on ethanol consumption in males and females, sex differences in the role of the DYN/KOR system in ethanol-related behaviors have not been extensively studied. The relationship between sex and interactions of stress and the DYN/KOR system may be of particular interest, especially when considering known sex differences in development of stress-related psychiatric disorders (Bangasser and Valentino, 2014). Given evidence for numerous sex differences in the sensitivity and function of DYN/KOR system (Chartoff and Mavrikaki, 2015), future work should address this considerable gap in the literature.

Mechanistic Studies

Although limited in scope, several studies have examined mechanisms that underlie DYN/KOR modulation of behavioral effects of ethanol. These studies have primarily focused on DYN/KOR adaptations in the nucleus accumbens and central nucleus of the amygdala. A number of studies have provided evidence that the DYN/KOR system exerts effects on the motivational effects of ethanol (and other drugs of abuse) in part by modulating mesolimbic dopamine (DA) release. Within the nucleus accumbens, KORs are located on DA terminals and inhibit DA release when activated (Svingos, 2011). Acute ethanol administration (via ip injection) increases DA release in the nucleus accumbens, and this effect is enhanced in KOR knockout mice or following pretreatment with a KOR antagonist (nor-BNI) in C57BL/6J mice (Zapata and Shippenberg, 2006). However, during operant ethanol self-administration, ethanol-induced DA release in the nucleus accumbens was blocked by nor-BNI, although a delayed DA increase was observed at the end of the ethanol access period (Doyon et al., 2006). Conversely, KOR activation has been shown to attenuate ethanol-induced DA release in the nucleus accumbens (Lindholm et al., 2007). The same report also demonstrated that nor-BNI increased extracellular DA in the nucleus accumbens of ethanol-treated rats, but not their saline-treated counterparts (Lindholm et al., 2007). More recent reports have shown a similar increase in sensitivity to KOR manipulation in subjects exposed to chronic ethanol. Specifically, chronic ethanol exposure and withdrawal in rodents (Rose et al., 2016; Karkhanis et al., 2016a) and monkeys (Siciliano et al., 2015) resulted in enhanced sensitivity of KORs in the nucleus accumbens. These studies demonstrated increased KOR agonist-induced suppression of DA transmission using fast scan cyclic voltammetry. This hypodopaminergic state is purported to contribute to dysphoria/negative affect associated with dependence and withdrawal, which may ultimately promote elevated ethanol consumption (Siciliano et al., 2015; Rose et al., 2016; Karkhanis et al., 2016a). A recent study using an optogenetic approach showed that activation of

subpopulations of dynorphin neurons in the nucleus accumbens mediate aversion (ventral shell region) and reward (dorsal shell region) (Al-Hasani et al., 2015). It will be important to determine whether ethanol differentially alters activity of these neuronal subpopulations, thereby shifting the balance between reward and aversion behaviors.

A few studies have examined the effects of ethanol on DYN/KOR function in the central nucleus of the amygdala. For example, electrophysiological studies showed that pharmacological manipulation of KORs altered acute ethanol-induced increases in GABA transmission (Kang-Park et al., 2013; Gilpin et al., 2014). Further, null mutant KOR mice showed greater ethanol-induced increases in GABA-mediated synaptic inhibition (Kang-Park et al., 2013). As previously described, adaptations indicative of greater KOR signaling and dynorphin peptide expression have been also observed during withdrawal from chronic ethanol vapor exposure (Kissler et al., 2014). These reported adaptations in the central nucleus of the amygdala are thought to mediate escalated drinking and behavioral measures of dysphoria/negative affect associated with ethanol dependence and withdrawal (Kissler et al., 2014; Kissler and Walker, 2016).

Future studies are needed to expand analysis of ethanol effects on the DYN/KOR system within the broader extended amygdala network (e.g., bed nucleus of the stria terminalis, basolateral amygdala). Additionally, as KORs residing on locus coeruleus noradrenergic (Kreibich et al., 2008) and dorsal raphe serotonergic (Land et al., 2009) neurons modulate ascending monoaminergic transmission, adaptations in these pathways likely influence motivational effects of ethanol as well as affective behaviors associated with chronic ethanol exposure and withdrawal. Within the prefrontal cortex, KORs have been shown to mediate glutamatergic and dopaminergic signaling (Tejeda et al., 2013; 2015). However, the role of DYN/KOR activity in cortical regions has not been studied with regard to ethanol-related behaviors. Finally, ethanol has been shown to engage a number of other stress-related neuropeptides (e.g., CRF, NPY, nociception, oxytocin), and there is some evidence demonstrating that these systems interact in contributing to motivational effects of ethanol (Koob, 2013; Martin-Fardon et al., 2010; Schank et al., 2012b). For example, anatomical (Marchant et al., 2007), neural (Kang-Park et al., 2015; Lam and Gianoulakis, 2011), and pharmacological (Bruchas et al., 2009; Funk et al., 2014; Land et al., 2008; Valdez et al., 2007) evidence indicates that DYN and CRF systems interact within brain reward and stress circuitry, but the nature of this interaction and how such effects may influence ethanol drinking, the ability of stress to modulate drinking, and enhanced motivation for ethanol along with negative affect associated with dependence is not fully understood. Thus, there is a great need for studies that probe mechanisms and circuitry that underlie adaptations and contributions of the DYN/KOR system in relation to motivational effects of ethanol.

Summary

A growing body of literature has emerged implicating a significant role for the DYN/KOR system in the motivational effects of ethanol. These studies have employed various models and experimental approaches. As highlighted in this review, results from this body of work are often inconsistent. This is likely due to numerous factors including differences in experimental parameters as well as limited analysis of the topic. Nevertheless, studies have

consistently supported the contention that the DYN/KOR system plays a significant role in contributing to dependence- and stress-induced increases in ethanol consumption. Figure 1 provides a conceptual overview of adaptations in the DYN/KOR system that may influence the rewarding effects of ethanol. Activation of this endogenous opioid peptide system has been shown to produce a negative hedonic state. Consequently, under conditions in which the DYN/KOR system is engaged (following stress or chronic ethanol exposure/withdrawal experience), the relative rewarding effects of ethanol may be enhanced, potentially contributing to elevated ethanol consumption. Under these circumstances, administration of a KOR antagonist may restore normal (basal) hedonic balance, thereby moderating ethanol reward and motivation to drink (Figure 1). More comprehensive analyses on behavioral and mechanistic levels are certainly needed to provide greater insight into how the DYN/KOR system is engaged and adapts to influence motivation to drink as well as emotional behaviors that trigger relapse. This information will be critical in spurring development of new agents that target DYN/KOR activity and hold promise as therapeutics for alcohol use disorders as well as comorbidity with affective disorders.

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Figure 1.

Conceptual schematic of adaptations in the DYN/KOR system in relation to motivational effects of ethanol. Under basal conditions, the rewarding effects of ethanol reflect an elevated (positive) hedonic state (gray line) whereby moderate levels of consumption are driven by positive reinforcement (i.e., drinking to achieve the rewarding effects of ethanol). Under these conditions, KOR antagonists do not affect basal hedonic state (or ethanol consumption). When the DYN/KOR system is activated pharmacologically via a KOR agonist (Panel A) or upregulated by stress or chronic ethanol exposure/withdrawal (Panel B),

the basal hedonic state is depressed. Ethanol consumption now results in a relatively greater positive shift in hedonic value (red line). Under these conditions, ethanol consumption is elevated because it is driven by a combination of positive reinforcement (drinking for rewarding effects) and negative reinforcement (drinking to alleviate dysphoria). KOR antagonists restore balance to the hedonic state such that the relative rewarding effects of ethanol are no longer potentiated (blue line). In this circumstance, ethanol consumption returns to moderate levels since drinking is no longer driven by negative reinforcement.

Table 1
Effects of pharmacological manipulation of KORs on ethanol conditioned reward and aversion

Subjects	Sex	Model	Drug/Dose/Timing	Effect of drug	References
DBA mice	n.r.	Ethanol CPP (2 g/kg, -5 min)	1 or 3 mg/kg U50,488 (10 min before ethanol)	blocked CPP	Logrip, Janak, and Ron, 2009
C57BL/6J mice	Male	Ethanol CPP (0.8 g/kg, -25 min) Stressed vs non-stressed	10 mg/kg U50,488 (90 min before ethanol) 10 mg/kg norBNI (24 hr before 1st conditioning)	potentiated CPP Stressed: blocked stress-potentiation of CPP Non-stressed: no effect on CPP	Sperling et al., 2010
C57BL/6J mice	Female	Ethanol CPP (2 g/kg, -15 min)	10 mg/kg norBNI (24 hr before 1st conditioning)	No change in standard CPP Greater state-dependent CPP	Nguyen et al., 2012
Sprague Dawley rats	Male	Ethanol CPP (0.3 g/kg, -30 min) Conditioned fear immed. before	1-3 mg/kg norBNI (4 hr before conditioning) 0.3-1 mg/kg U50,488 (immed. before conditioning)	3 mg/kg norBNI increased stress-induced ethanol CPP 0.3 and 1 mg/kg U50,488 reduced stress-induced ethanol CPP	Matsuzawa et al., 1999
Sprague Dawley rats	Male	Ethanol CPP (0.3 g/kg, -30 min) Footshock immed. before	1-3 mg/kg norBNI (4 hr before conditioning)	No effect of norBNI on stress-induced ethanol CPP U50,488 tended to reduce (n.s.) stress-induced ethanol CPP	Matsuzawa et al., 1998
Sprague Dawley rats	Male	Ethanol CTA (1 - 2 g/kg) Stressed vs non-stressed Adolescent and adult rats	10 mg/kg norBNI (24 hr before test)	No effects of norBNI in adolescents or non-stressed adults NorBNI blunted ethanol induced CTA in stressed adult rats	Anderson et al., 2013
Fischer/Lewis rats	Female	Ethanol CTA (1.25 g/kg)	1 mg/kg norBNI (24 hr before conditioning)	No change in ethanol CTA	Roma et al., 2008

n.r., not reported; CPP, conditioned place preference; CTA, conditioned taste aversion; norBNI, nor-binaltorphimine; n.s., not significant

Table 2

Effects of pharmacological manipulation of KORs on homecage ethanol drinking

Effects of KOR agonism					
Subjects	Sex	Drinking model	Drug/Timing	Site	Effect on drinking
Wistar rats	Male	2 bottle choice (24 hr)	enadoline	systemic (minipump)	Increase
Wistar rats	Male	2 bottle choice (24 hr)	U50,488 (0 min)	systemic (ip) × 5 days	Decrease (day 1 only)
Wistar rats	Male	2 bottle choice (24 hr)	dynorphin 1-17 (-15 min)	systemic (sc)	Decrease
Sprague-Dawley rats	Male	2 bottle choice (12 hr)	U50,488 (0 min)	PF-LH	Decrease (1st 2 hr)
Sprague-Dawley rats	Male	2 bottle choice (12 hr)	U50,488 (-30 min)	PVN	Decrease
Sprague-Dawley rats	Male	2 bottle choice (12 hr)	U50,488 (-30 min)	NAC	No change
Lewis rats	Male	2 bottle choice (2 hr)	U50,488 (-15 min)	systemic (ip)	Decrease
Alko-Alcohol rats	Male	2 bottle choice (90 min)	U50,488 (0 min)	VP	No change
C57BL/6J mice	Male	2 bottle choice (24 hr)	U50,488 (-24 hr)	systemic (ip)	Increase (short term)
C57BL/6J mice	Male	2 bottle choice (2 hr)	U50,488 (-30 min)	systemic (ip)	Increase
C57BL/6J mice	Male	1 bottle (1 hr)	U50,488 (-60 min)	systemic (ip) × 5 days	Increase (all days)
C57BL/6J mice	Male	2 bottle choice (24 hr)	U50,488 (0 min)	systemic (sc) daily	Increase in socially stressed mice only
C57BL/6J mice	Male	2 bottle choice (24 hr)	MSB (-30 min)	systemic (ip)	Decrease at 4 hr
C57BL/6J mice	Female	intermittent access	MSB (-30 min)	systemic (ip)	Decrease at 4 hr
C57BL/6J mice	Male	1 bottle (4 hr)	MSB (-30 min)	systemic (ip)	No change
C57BL/6J mice	Female	1 bottle (4 hr)	MSB (-30 min)	systemic (ip)	No change
Effects of KOR antagonism					
Subjects	Sex	Drinking model	Drug/Timing	Site	Effect
Wistar rats	Male	2 bottle choice (24 hr)	MR-2266-BS	systemic (sc)	Decrease
Wistar rats	Male	2 bottle choice (24 hr)	norBNI	systemic (sc)	No change
Lewis rats	Male	2 bottle choice (24 hr)	norBNI	systemic (sc)	Increase (high drinkers only)
Lewis rats	Female	2 bottle choice (2 hr)	norBNI (-30 min)	systemic (sc)	No change
Long Evans rats	Male	2 bottle choice (24 hr intermittent)	norBNI (-24 hr)	systemic (ip)	Decrease (1st 30 min, stressed only)
Alko-Alcohol rats	Male	2 bottle choice (90 min)	norBNI (0)	VP	No change
P-rats	Female	2 bottle choice (24 hr)	LY2456302 (-60 min)	systemic (po)	Decrease (during 12-hr dark cycle)
Reference					
					Holter et al., 2000
					Nestby et al., 1999
					Sandi et al., 1988
					Chen et al., 2013
					Barson et al., 2010
					Barson et al., 2009
					Lindholm et al., 2001
					Kemppainen et al., 2012
					Sperling et al., 2010
					Rose et al., 2016
					Anderson et al., 2016
					Kudryavtseva et al., 2006
					Zhou et al., 2017
					Zhou et al., 2017
					Zhou et al., 2017
					Zhou et al., 2017
					Zhou et al., 2017
					Zhou et al., 2017
					Sandi et al., 1990
					Holter et al., 2000
					Mitchell et al., 2005
					Lindholm et al., 2001
					Karkhanis et al., 2016
					Kemppainen et al., 2012
					Rorick-Kehn et al., 2014

Effects of KOR agonism						
Subjects	Sex	Drinking model	Drug/Timing	Site	Effect on drinking	Reference
P-rats	Male	2 bottle choice (30 min)	compound 5 (-30 min) **partial mu agonist	systemic (sc)	Decrease	Cashman and Azar, 2014
Sprague-Dawley rats	Male	1 bottle (30 min)	norBNI (-24 hr)	systemic (sc)	Increase	Morales et al., 2014
Sprague-Dawley rats	Female	1 bottle (30 min)	norBNI (-24 hr)	systemic (sc)	Decrease	Morales et al., 2014
Rhesus monkeys	Male	2 bottle choice (3 hr)	norBNI(-30 min)	systemic (im)	Decrease	Williams and Woods, 1998
C57BL/6J mice	Male	1 bottle (1 hr)	LY2444296 (-30 min)	systemic (ip)	Decrease (stressed + ethanol dependent only)	Anderson et al., 2016
C57BL/6J mice	Male	2 bottle choice (2 hr)	norBNI (-24 hr)	systemic (ip)	Decrease (ethanol-dependent only)	Rose et al., 2016
C57BL/6J mice	Male	2 bottle choice (24 hr)	norBNI (-24 hr)	systemic (ip)	Decrease (stressed only)	Sperling et al, 2010
C57BL/6J mice	n.r.	2 bottle choice (18 hr)	norBNI (-30 min)	systemic (ip)	Decrease	Logrip et al., 2008
C57BL/6J mice	Male	2 bottle choice (24 hr)	norBNI (-24 hr)	systemic (ip)	Decrease at 4 hr	Zhou et al., 2017
C57BL/6J mice	Female	intermittent access	norBNI (-24 hr)	systemic (ip)	No change	Zhou et al., 2017

PF-LH, perifornical hypothalamus; PVN, paraventricular hypothalamus; NAc, nucleus accumbens; VP, ventral pallidum; ip, intraperitoneal; sc, subcutaneous; norBNI, nor-binaltorphimine; MSB, Mesyl Salvinorin B

Table 3

Effects of pharmacological manipulation of KORs on operant self-administration

Effects of KOR agonism					
Subjects	Sex	Drinking model	Drug	Site	Effect on self-administration
Wistar rats	Male	Operant self-admin (23 hr)	enadoline (–15 min)	systemic (ip)	Decrease (1st hr only)
Wistar rats	Male	Operant self-admin (30 min)	U50,488 (–10 min)	systemic (ip)	Induced reinstatement
Long Evans rats	Male	Operant self-admin (1 hr)	U50,488 (–30 min)	systemic (ip)	Induced reinstatement
Long Evans rats	Male	Operant self-admin (20 min)	U50,488 (–20 min)	systemic (ip)	Decrease
P-rats	Male	Operant self-admin (20 min)	U50,488 (–20 min)	systemic (ip)	Decrease
Effects of KOR antagonism					
Subjects	Sex	Drinking Model	Drug	Site	Effect on self-administration
Wistar rats	Male	Operant self-admin (30 min)	norBNI	icv	Decrease (EtOH-dependent only)
Wistar rats	Male	Operant self-admin (30 min)	norBNI	systemic (sc)	Decrease (EtOH-dependent only)
Wistar rats	Male	Operant self-admin (30 min)	norBNI	CeA	Decrease (EtOH-dependent only)
Wistar rats	Male	Operant self-admin (30 min)	norBNI	NAC	Decrease (EtOH-dependent only)
Wistar rats	Male	Operant self-admin (30 min)	norBNI	icv	Decreased cue-induced elevated intake
Wistar rats	Male	Operant self-admin (30 min)	norBNI (–2, 24, 48 hr)	systemic (ip)	Decrease at 2 hr only
Wistar rats	Male	Operant self-admin (30 min)	JDTric (–2, 24, 48 hr)	systemic (ip)	Decrease at 2 hr only
Wistar rats	Male	Cue- and stress-induced reinstatement	JDTric (–2, 24, 48 hr)	systemic (ip)	Decreased cue- but not stress-induced reinstatement
Long Evans rats	Male	U50,488- and YOH-induced reinstatement	norBNI (–2, 24 hr)	systemic (ip)	Decreased U50,488- and YOH-induced reinstatement at 2 hr
Long Evans rats	Male	Operant self-admin (45 min)	norBNI (–20 hr)	systemic (sc)	No change
P-rats	Male	Operant self-admin (30 min)	compound 5 (–30 min) ***partial mu agonist	systemic (sc)	Decrease
P-rats	Male	Operant progressive ratio responding	LY2456302	systemic (po)	Decreased lever presses, breakpoints, and intake
P-rats	Female	Operant self-admin (60 min), extinction, spontaneous recov and relapse	JDTric (–14 days before spont recov)	systemic (sc)	Blocked spontaneous recov and relapse

CeA, central nucleus of amygdala; NAC, nucleus accumbens; VP, ventral pallidum; icv, intracerebroventricular infusion; sc, subcutaneous; ip, intraperitoneal; po, per os (oral); norBNI, nor-binaltorphimine; YOH, yohimbine

Table 4

Effects of genetic manipulation of DYN/KOR on ethanol-related behaviors

Ethanol-conditioned reward and aversion				
Subjects	Sex	Model	Effect of genetic deletion	References
Pdyn KO mice	Female	Ethanol CPP (2 g/kg, ip)	No difference in standard CPP Greater state-dependent ethanol CPP	Nguyen et al., 2012
Pdyn KO mice	Male	Ethanol CPP (2 g/kg, po)	Greater ethanol CPP	Femenia and Manzanaraes, 2012
Pdyn KO mice	Male	Ethanol CPP (2 g/kg, ip)	No difference in CPP	Blednov et al., 2006
	Female		No difference in CPP	Blednov et al., 2006
Pdyn KO mice	Male	Ethanol CTA (2.5 g/kg, ip)	No difference in CTA	Blednov et al., 2006
	Female		No difference in CTA	Blednov et al., 2006
Homecage ethanol drinking				
Subjects	Sex	Drinking model	Effect of genetic deletion	References
KOR KO mice	Male	2 bottle choice (24 hr, 12%)	Reduced intake and preference	Kovacs et al., 2005
	Female	2 bottle choice (24 hr, 12%)	Reduced intake and preference	Kovacs et al., 2005
Pdyn KO mice	Male	2 bottle choice (24 hr, 2–8%)	Increased intake and preference	Femenia and Manzanaraes, 2012
Pdyn KO mice	Male	2 bottle choice (24 hr, 3–12%)	No difference	Blednov et al., 2006
KOR KO mice	Female	2 bottle choice (24 hr, 3–12%)	Reduced intake and preference	Blednov et al., 2006
Pdyn KO mice	Male	2 bottle choice (24 hr, 8%)	Increased intake and preference	Racz et al., 2013
	Female	2 bottle choice (24 hr, 8%)	Increased intake but not preference	Racz et al., 2013
	Male	2 bottle choice before/after footshock	No stress-induced increase	Racz et al., 2013
	Female	2 bottle choice before/after footshock	Increased intake 72 hr after stress	Racz et al., 2013
KOR KO mice	Female	2 bottle choice (24 hr, 3–15%)	Reduced intake and preference	Van't Veer et al., 2016
KOR KO mice	Female	Intermittent access (15%)	Reduced intake and preference	Van't Veer et al., 2016
KOR KO mice	Female	Binge-like drinking (DID; 20%)	No difference	Van't Veer et al., 2016
Pdyn KO mice	Male	2 bottle choice (24 hr, 3–10%)	No difference	Sperling et al., 2010
	Male	2 bottle choice (24 hr, 10%)	No stress-induced increase	Sperling et al., 2010

Pdyn, prodynorphin; KO, knockout; CPP, conditioned place preference; po, per os (oral); CTA, conditioned taste aversion; DID, drinking-in-the-dark