

Review

# The Role of the Dynorphin/Kappa Opioid Receptor System in the Actions of Alcohol

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**Abstract:** Alcohol use disorder (AUD) represents major public and socioeconomic issues. Alcohol exerts its pharmacological effects by altering different neurotransmitter systems, such as g-aminobutyric acid (GABA), glutamate, opioids, etc. Recent evidence suggests that the dynorphin (DYN)/kappa opioid receptor (KOR) system mediates the negative affective states associated with alcohol withdrawal. This system is also involved in stress-mediated alcohol intake in alcohol-dependent subjects. The DYN/KOR system probably exerts its action in the central nucleus of the amygdala (CeA) to mediate the negative affective states associated with alcohol withdrawal. This article aims to review the current literature regarding the role of the DYN/KOR system in the actions of alcohol. We first review the literature regarding the effect of alcohol on the level of the peptide and its receptor, and the role of the endogenous DYN/KOR system in alcohol reward and negative affective states associated with alcohol withdrawal is then discussed. We also review the literature regarding the effects of KOR ligands on these processes.

**Keywords:** alcohol; dynorphin; kappa opioid receptor; DYN/KOR; anxiety; depression; negative affective states; withdrawal; microdialysis; dynorphin-immunoreactivity



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## 1. Introduction

Alcohol use disorder (AUD) is a major public health problem in the United States and around the globe. It is marked by the lack of control to stop or reduce alcohol intake. People who drink excessively over a long time may suffer from the long-term effects of alcohol, such as unsafe behaviors, hepatitis, fibrosis, and cirrhosis [1]. Moreover, alcoholism can cause cancers and cardiovascular problems [2]. Alcohol use and abuse are the seventh-leading cause of premature death and disability worldwide. In the United States, alcohol is considered the third-leading preventable cause of death [3]. The National Survey on Drug Use and Health (NSDUH, 2019) (<https://www.samhsa.gov/data/report/2019-nsduh-annual-national-report>, accessed on 3 October 2022) reports that 14.5 million people aged 12 and older have AUD. This number includes 9.0 million men and 5.5 million women.

The socioeconomic impact of AUD is also tremendous. The annual social expenses of AUD are estimated to be at least USD 148 billion [4]. Therefore, more research is needed to characterize new targets to develop medications to treat AUD effectively and safely. Any advancement in this field would be highly desirable to help those suffering from AUD and decrease the cost to society.

The effects of alcohol differ from person to person, based on various factors such as how much and how frequently a person drinks alcohol. Additionally, it depends on age, gender, health status, and family history of alcoholism [5]. Binge drinking, defined as increased alcohol consumption over a relatively short time, is a regular pattern of alcohol

consumption in the United States and has negative public health and socioeconomic and behavioral consequences (Centers for Disease Control and Prevention, <https://search.cdc.gov/search/?query=Alcohol&dpag=1>, accessed on 3 October 2022). However, while not all people who binge drink are addicted to alcohol, binge drinking is a significant element in the progression of AUD. Chronic alcohol use has been shown to rewire the reward and anti-reward neurocircuitries, leading to negative affective states, which then serve as a negative reinforcer and result in relapse (for reviews, see [6–9]).

Withdrawal symptoms are one of the distinguishing signs of alcoholism, and it is frequently characterized by a decrease in physiological responses and increased negative affective states [10–12]. Withdrawal signs are categorized into somatic and affective signs. Given that the somatic signs of withdrawal last for shorter periods than the motivational, affective, and cognitive deficits associated with alcohol withdrawal, a single pharmacotherapy that reduces alcohol consumption during both acute withdrawal and protracted abstinence would be an ideal goal for the treatment of AUD [13,14]. Increasing evidence suggests that the dynorphin (DYN)/kappa opioid receptor (KOR) system is involved in the action of alcohol and negative affective states associated with alcohol withdrawal, particularly in subjects dependent on alcohol and undergoing stressful conditions.

DYN is derived from a precursor known as prodynorphin (pDYN), encoded by the preprodynorphin (ppDYN) gene. DYN exists in many forms, including DYN A and DYN B (for a review, see [15]). DYN possesses a high affinity toward the KOR. The peptide and its corresponding receptor are found in the cortex, amygdala, striatum, thalamus, hypothalamus, midbrain, hippocampus, pons, and medulla ([16,17]; for a review, see [18]). Other studies show that the highest expression of KORs lies on the mesolimbic dopaminergic neurons, projecting from the ventral tegmental area (VTA) to the nucleus accumbens (NAc). Indeed, DYN is known to regulate dopaminergic tone and to produce aversion and dysphoria ([19–21]; for a review, see [22]).

Changes in the level of DYN have been reported following treatment with addictive drugs. For example, the DYN level increases in response to alcohol administration and following withdrawal from alcohol [23,24], which is thought to mediate the negative affective states associated with alcohol withdrawal ([10–12]; for a review, see [15]). This article reviews the literature regarding the role of the DYN/KOR system in the acute and chronic actions of alcohol. We first provide evidence for the action of alcohol on the DYN/KOR system and then describe the results of the studies showing that this system regulates the actions of alcohol.

## 2. Materials and Methods

A PubMed search was conducted using the keywords: Alcohol, Opioids, Alcohol Use Disorders, Dynorphin, Kappa Opioid Receptor, DYN/KOR, Anxiety, Depression, Negative Affective States, Withdrawal, Consumption, Elevated Plus Maze, Forced Swim Test, Microdialysis, Dynorphin-immunoreactivity (DYN-IR). We selected a total of 96 peer-reviewed articles related to the role of the DYN/KOR system in the action of alcohol, reviewed 74 (referenced here), and summarized 35 of those (Table 1).

**Table 1.** Summary of articles reviewed and included in this article.

Nr.	Study by	Paper Title	Subject	Sex	Drug Dose Route	Category	Brain Area/Peptide/Peptide Precursors, etc.	Findings
1	Anderson RI, Becker HC (2017) [6]	Role of the dynorphin/kappa opioid receptor system in the motivational effects of ethanol	N/A	N/A	N/A	Role of DYN/KOR system on stress-mediated alcohol intake and withdrawal	NAC, CeA, U50,488, nor-BNI, MSB, LY2456302 enadoline, JDITic	Acute and chronic ethanol exposure upregulates the DYN/KOR system; KOR antagonists reduce the negative effects of stress and ethanol withdrawal.
2	Anderson RI, Lopez MF, Griffin WC, et al. (2019) [25]	Dynorphin-kappa opioid receptor activity in the central amygdala modulates binge-like alcohol drinking in mice	C57BL/6 J mice	Male	IP	Effect of DYN/KOR system on binge drinking	U50488, nor-BNI, CNO, CeA	U50,488 increased binge-like drinking, but nor-BNI into the CeA reduced drinking in the DID model.
3	Bazov I, Sarkisyan D, Kononenko O, et al. (2018) [26]	Dynorphin and $\kappa$ -opioid receptor dysregulation in the dopaminergic reward system of humans with AUDs	Humans (post-mortem)	Male	N/A	Effect of chronic alcohol use on pDYN and OPRK1 gene expression	NAC, D1DR, D2DR	pDYN and OPRK1 showed a transcriptionally coordinated pattern between humans with AUDs and controls. Downregulation of DRD1 but not DRD2 expression in those with AUDs. Expression of DRD1 and DRD2 correlated with pDYN and OPRK1.
4	Becker HC (2017) [7]	Influence of stress associated with chronic alcohol exposure on drinking	N/A	N/A	N/A	Stress-ethanol consumption and peptides	CRF, DYN, nociceptin, NPY, oxytocin, BNST, CeA	Discusses the role of peptides involved in stress (CRF, DYN, and others) and anti-stress neuropeptides (nociceptin, NPY, oxytocin) in negative affective states and motivation for alcohol consumption.
5	Blednov YA, Walker D, Martinez M, Harris RA (2006) [27]	Reduced alcohol consumption in mice lacking preprodynorphin	C57Bl/6J x 129/SvEv-Tac Mice (ppDYN KO and WT)	Male and Female	N/A	Effect of preprodynorphin gene on alcohol consumption	ppDYN, KOR	Deletion of preprodynorphin reduced alcohol intake in female mice, possibly caused by the increased rewarding effect of alcohol.
6	Haun HL, Griffin WC, Lopez MF, Becker HC, (2020) [28]	Kappa opioid receptors in the bed nucleus of the stria terminalis regulate binge-like alcohol consumption in male and female mice	C57BL/6J Mice	Male/Female	IP, PO	Effect of KOR on binge drinking of alcohol in BNST in both male/female mice	BNST, nor-BNI, U50,488	DYN/KOR system in the BNST plays a role in binge drinking in both male and female mice.
7	Logrip ML, Janak PH, Ron D (2008) [29]	Dynorphin is a downstream effector of striatal BDNF regulation of ethanol intake	C57BL/6J mice Long Evans rats Sprague-Dawley pups	not stated	PO	BDNF receptor expression in response to alcohol consumption in the two-bottle choice	ppDYN, KOR	Exposure of striatal neurons led to TrkB activation, MAP kinase activation, and ppDYN expression. KOR was needed for the BDNF reduced alcohol intake.
8	Logrip ML, Janak PH, Ron D (2009) [30]	Blockade of ethanol reward by the kappa opioid receptor agonist U50,488H	DBA/2J (DBA) mice	Not Indicated	IP	KOR activation blocks ethanol reward	U50488H	Low, non-aversive doses of U50,488H blocked the ethanol-induced place preference and condition hyperlocomotion.
9	Fallon JH, Leslie FM (1986) [16]	Distribution of dynorphin and enkephalin peptides in the rat brain	Albino mice	Male and Female	N/A	Peptide localization	DM, NAC, VMN, PVN, SON, VTA, locus coeruleus	DYN and enkephalin are located in regions that control extrapyramidal cell function, homeostasis, and sensory perception.
10	Gillett K, Harshberger E, Valdez GR (2013) [10]	Protracted withdrawal from ethanol and enhanced responsiveness stress: regulation via the dynorphin/kappa opioid receptor system	Wistar rats	Male	IP	DYN/KOR system effect on stress	nor-BNI, U50488	Ethanol-dependent rats showed increased anxiety compared to controls, blocked by nor-BNI. U50,488 also increased anxiety at the highest dose.

**Table 1.** *Cont.*

Nr.	Study by	Paper Title	Subject	Sex	Drug Dose Route	Category	Brain Area/Peptide/Peptide Precursors, etc.	Findings
11	Gilpin NW, Herman MA, Roberto M (2015) [31]	Kappa opioid receptor activation decreases inhibitory transmission and antagonizes alcohol effects in rat central amygdala	Sprague-Dawley rats	Male	N/A	KOR effect on inhibitory control and alcohol consumption in CeA	DYN, CeA, U69593, nor-BNI, CTAP, GABA	DYN or U69593 superfusion in CeA dose-dependently lowered IPSPs, blocked by nor-BNI. nor-BNI alone increased GABAergic transmission, suggesting its involvement in the KOR system. Ethanol superfusion increased IPSPs, reversed upon pretreatment with KOR agonists.
12	Jamensky NT, Gianoulakis C (1997) [32]	Content of dynorphins and kappa opioid receptors in distinct brain regions of C57BL/6 and DBA/2 mice	C57BL/6 and the DBA/2 mice	Male	N/A	Strain/site-related differences between DYN and KOR	DYN A 1-8, DYN A 1-13, pDYN mRNA, VTA, NAc, PAG, septum	C57BL/6 mice had more KOR binding sites and dynorphin A 1-13 in the amygdala and DYN A 1-8 in the VTA. DBA/2 mice had more KOR binding sites, pDYN mRNA, and DYN A 1-13/1-8 in NAc and the septum. DBA/2 mice also had more KOR in the PGA and DYN A 1-13 and DYN A 1-8 in the caudate putamen.
13	Jarjour S, Bai L, Gianoulakis C (2009) [33]	Effect of acute ethanol administration on the release of opioid peptides from the midbrain, including the ventral tegmental area	Sprague-Dawley rats	Male	IP	Effect of ethanol on the release of opioid peptides from the midbrain/VTA	DYN A 1-8, beta-endorphin, met-enkephalin, VTA, midbrain	Ethanol may increase beta-endorphin release at low-to-medium doses in the midbrain/VTA to reinforce alcohol consumption.
14	Jarman SK, Haney AM, Valdez GR (2018) [34]	Kappa opioid regulation of depressive-like behavior during acute withdrawal and protracted abstinence from ethanol	Male	Wistar rats	IP	KOR system on chronic ethanol abstinence	nor-BNI	Ethanol-dependent subjects showed decreased mobility, indicative of a depressive-like state, attenuated by nor-BNI.
15	Karkhanis AN, Al-Hasani R (2020) [15]	Dynorphin and its role in alcohol use disorder	N/A	N/A	N/A	Role of DYN in AUD	VTA, NAc, BNST, KOR	Reviews literature regarding the role of DYN in AUD.
16	Lam MP, Marinelli PW, Bai L, Gianoulakis C (2008) [35]	Effects of acute ethanol on opioid peptide release in the central amygdala: an in vivo microdialysis study	Sprague-Dawley rats	Male	IP	Acute ethanol on opioid peptide release in the CeA	met-enkephalin, $\beta$ -endorphin, DYN A1–8, CeA	Ethanol (2.8 g/kg) increased $\beta$ -endorphin and DYN A 1–8 release. No effect on met-enkephalin release.
17	Kissler JL, Sirohi S, Reis DJ, et al. (2014) [36]	The one-two punch of alcoholism: role of central amygdala dynorphins/kappa opioid receptors	Wistar rats	Male	Intracranial	DYN/KOR system in CeA	CeA, nor-BNI, CTOP/naltrindole or nalmefene, DYN A	Ethanol-dependent rats showed increased alcohol consumption, negative affective states, and DYN expression in the amygdala compared to controls. nor-BNI reduced alcohol consumption in dependent rats, and a MOR antagonist/partial KOR agonist affected both dependent rats and controls.
18	Kissler JL, Walker BM (2016) [37]	Dissociating motivational from physiological withdrawal in alcohol dependence: role of central amygdala $\kappa$ -opioid	Male	Wistar	IV	KOR system in CeA	nor-BNI, CeA	nor-BNI in the CeA reduced escalation of alcohol consumption in dependent rats without affecting signs of withdrawal.

**Table 1.** *Cont.*

Nr.	Study by	Paper Title	Subject	Sex	Drug Dose Route	Category	Brain Area/Peptide/Peptide Precursors, etc.	Findings
19	Koob GF (2014) [8]	Neurocircuitry of alcohol addiction: synthesis from animal models	N/A	N/A	N/A	Decreased reward function and increased activity of the stress circuit	Dopamine, CRF, opioids, NAC, amygdala	Neuronal adaptation following chronic alcohol use leads to decreased dopaminergic neurotransmission and increased CRF in the amygdala.
20	Koob GF (2020) [9]	Neurobiology of opioid addiction: synthesis process, hyperkatifeia, and negative reinforcement	N/A	N/A	N/A	Opponent processes and negative reinforcement on opioid usage	BNST, CeA, DS, dlPFC, GP, NAC, OFC, PAG, Orexin, CRF, NPY, nociceptin, oxytocin, NE, DYN, vasopressin, glucocorticoids	Neuronal plasticity of the pain system via drug intake can become sensitive with repeated withdrawal promoting compulsive seeking behavior.
21	Koob GF, Volkow ND (2010) [14]	Neurocircuitry of addiction	N/A	N/A	N/A	Addiction effects on neurocircuitry	Hippocampus, prefrontal cortex, thalamus, CeA, BNST, dorsal striatum, VTA, DYN	Reviews the literature on neurocircuitry on the development of addiction
22	Kovacs KM, Szakall I, O'Brien D, et al. (2005) [38]	Decreased oral self-administration of alcohol in kappa opioid receptor knockout mice	C57BL/6ORL Mice KO vs. WT vs. HET	Male and Female	N/A (oral self-administration)	Effect of KOR on food intake	NAC, DA, DYN	Male KOR KO mice show decreased alcohol and saccharin but increased quinine preference than wild-type (WT). Female KOR KO mice also drank less than WT and HET mice.
23	Lindholm S, Ploj K, Franck J, Nylander I (2000) [23]	Repeated ethanol administration induces short- and long-term changes in enkephalin and dynorphin tissue concentrations in rat brain	Sprague-Dawley rats	Male	IP	Effect of ethanol on enkephalin and DYN tissue contents in rat brain	DYN B 1-13	Soon after the last ethanol dose, the DYN B tissue was reduced in the cingulate cortex, while MEAP tissue was reduced. Both DYN B and the MEAP concentrations were increased in the PAG. DYN B elevated in the NAc after the last ethanol dose.
24	Lindholm S, Werme M, Brené S, Franck J (2001) [39]	The selective [kappa]- opioid receptor agonist U50,488H attenuates voluntary ethanol intake in the rat	Male	Lewis Rats	IP	KOR system on ethanol intake	U50,488H, nor-BNI	U50,488H dose-dependently reduced ethanol consumption in rats. The effect of the highest dose of U50,488H was reduced by pretreatment with nor-BNI.
25	Marinelli PW, Lam M, Bai L, Quirion R, Gianoulakis C, (2006) [40]	Kappa 1 receptor mRNA distribution in the rat CNS: comparison to kappa receptor binding and prodynorphin mRNA	Sprague-Dawley rats	Male	IP	Effect of alcohol on DYN A 1-8 in NAc	DYN A 1-8, NAc	Compared to controls, alcohol (1.6 and 3.2 g/kg) caused a short-term increase in DYN A 1-8 and a more pronounced effect with a higher dose (3.2 g/kg).
26	Mitchell JM, Liang MT, Fields HL (2005) [41]	A single injection of the kappa opioid antagonist nor-binaltorphimine increases ethanol consumption in rats	Lewis rats	Male	Sub-Cut	Effect of nor-BNI on ethanol consumption	nor-BNI	Injection of nor-BNI increases ethanol consumption in high drinkers without affecting alcohol reward in the place conditioning paradigm.
27	Nguyen K, Tseng A, Marquez P, Hamid A, Lutfy K, (2012) [42]	The role of endogenous dynorphin in ethanol-induced state-dependent CPP	C57BL/6 mice	Male and Female	IP	Role of DYN/KOR system state-dependent conditioned place preference	nor-BNI	A similar CPP was seen in mice pDYN KO, WT control, and WT treated with n-BNI. However, these pDYN KO mice showed a greater CPP response after an ethanol challenge.

Table 1. Cont.

Nr.	Study by	Paper Title	Subject	Sex	Drug Dose Route	Category	Brain Area/Peptide/Peptide Precursors, etc.	Findings
28	Ploj K, Roman E, Gustavsson L, Nylander I (2000) [43]	Basal levels and alcohol-induced changes in nociceptin/orphanin FQ, dynorphin, and enkephalin levels in C57BL/6J mice	C57BL/6J and DBA/2J mice	Male	N/A	Nociceptin/orphanin FQ (N/OFQ) system in alcohol drinking behavior	N/OFQ, DYN, and enkephalin, DYN B, MEAP, amygdala, frontal cortex, hypothalamus, periaqueductal gray, striatum, VTA, kainic acid, NMDA	There are differences in the peptide levels between C57BL/6J and DBA/2J mice that explain differences in ethanol consumption.
29	Sirohi S, Aldrich JV, Walker BM (2016) [44]	Species differences in the effects of the $\kappa$ opioid receptor antagonist zyklophin	Wistar rats and C57BL/6J mice	Male	ICV/SC	Zyklophin	Zyklophin	Escalated alcohol self-administration in dependent subjects is due to a dysregulated DYN/KOR system. Extended durations of action reduce escalated alcohol consumption in alcohol-dependent animals. Self-administration or locomotor activity in either exposure condition was not impacted by zyklophin.
30	Sperling RE, Gomes SM, Sypek EI, Carey AN, McLaughlin JP (2010) [45]	Endogenous kappa-opioid mediation of stress-induced potentiation of ethanol-conditioned place preference and self-administration	C57Bl/6J mice (pDYN KO vs. WT)	Male	IP	Ethanol-CPP	U50488, nor-BNI	Mice undergoing forced swim stress (FSS) before ethanol showed a large increase in the potentiation of CPP compared to unstressed mice. U50,488 pretreatment attenuated this increase, while nor-BNI reversed it. FSS increased ethanol consumption. pDYN KO and WT showed no differences in ethanol consumption.
31	Valdez GR, Harshberger E (2012) [12]	K opioid regulation of anxiety-like behavior during acute ethanol withdrawal	Wistar rats	Male	IP	KOR regulation during ethanol withdrawal	nr-BNI, U50,488	A significant decrease in open-arm exploration was observed in rats experiencing ethanol withdrawal, an effect blocked by nor-BNI. Decreases were also shown in open-arm exploration following injections with the KOR agonist, U50,488.
32	Van't Veer A, Smith KL, Cohen BM, Carlezon WA, Jr., Bechtholt AJ (2015) [46]	Kappa opioid receptors differentially regulate low and high levels of ethanol intake in female mice	KOR KO vs. WT using Cre/Lox strategy	Female	N/A	Effect of KOR containing DA neurons on ethanol intake	DA, VTA	Using a Cre-Lox system to create KOR knockout in mice and assess ethanol preference, the authors showed reduced alcohol consumption in the mutant mice. However, this effect was less obvious when EtOH intake was higher.
33	Walker BM, Koob GF (2008) [47]	Pharmacological evidence for a motivational role of kappa opioid systems in ethanol dependence	Wistar rats	Male	ICV	Effect of KOR on ethanol consumption in dependent animals	Naltrexone, nalmefene, nor-BNI	Nalmefene and naltrexone dose-dependently decreased ethanol self-administration in dependent and non-dependent mice, but in ethanol-dependent mice, nalmefene suppressed ethanol intake much more than naltrexone. nor-BNI decreased ethanol-dependent alcohol consumption but did not affect non-dependent mice.

**Table 1.** *Cont.*

Nr.	Study by	Paper Title	Subject	Sex	Drug Dose Route	Category	Brain Area/Peptide/Peptide Precursors, etc.	Findings
34	Walker BM, Valdez GR, McLaughlin JP, Bakalkin G (2012) [48]	Targeting dynorphin/kappa opioid receptor systems to treat alcohol abuse and dependence	N/A	N/A	N/A	Role of DYN/KOR system in alcohol addiction	DYN/KOR, U50,488, U69,593, salvinorin A, nor-BNI, $\alpha$ -NE, CeA, $\beta$ END/MOR, ENK/DOR	Discusses the role of KOR and DYN in drinking behavior, negative affective states, and disruption in cognition.
35	Zapata A, Shippenberg TS (2006) [49]	Endogenous kappa opioid receptor systems modulate the responsiveness of mesoaccumbal dopaminergic neurons to ethanol	C57BL/6J mice (KOR and KOR gene) KO vs. WT	Male	SC	Effect of KOR on DA activation in NAc	DA, NAC, nor-BNI	Acute ethanol increased NAc DA levels In KOR gene KO more than WT counterparts. A similar result was obtained in animals treated with nor-BNI; repeated ethanol exposure decreased these changes.



### 3. Results

The review of the current literature revealed that alcohol affects the endogenous DYN/KOR system, and this system plays a functional role in the actions of alcohol, particularly in affective signs of alcohol withdrawal in subjects dependent on alcohol and exposed to stress. We first discuss the effect of alcohol on the level of DYN and KORs in different brain areas. We then discuss the involvement of the DYN/KOR system in the action of alcohol, followed by the neuroanatomical sites of action of the endogenous DYN. We also discuss the impact of gender/sex and age on the role of this system in the action of alcohol.

#### 3.1. Effects of Alcohol on the DYN/KOR System

Initial work by Jamensky and Gianoulakis sought to determine whether there were variations in KOR density and the level of pDYN mRNA and DYN in different brain areas of alcohol-preferring C57BL/6J mice and alcohol-avoiding DBA/2 mice [32]. It was reported that C57BL/6J mice exhibited increased alcohol consumption and had more KOR binding sites and DYN A (1–13) in the amygdala and DYN A (1–8) in the VTA. In contrast, the DBA/2 mice that consume less alcohol expressed considerably higher KOR binding sites, pDYN mRNA, as well as DYN A (1–13), and DYN A (1–8) in the NAc and septum [32]. Furthermore, DBA/2 mice had higher levels of KOR in the periaqueductal gray (PAG) and DYN A (1–13), and DYN A (1–8) in the caudate putamen. The authors concluded that as the increased stimulation of accumbal KOR is linked to decreased dopamine release and aversive states, the higher levels of KOR, pDYN mRNA, and DYN peptides in the limbic regions of the DBA/2 mice may explain why these mice consume less alcohol than C57BL/6J mice [32]. However, further studies are needed to prove this concept, as mice lacking DYN or KOR self-administer less alcohol than their wild-type controls [27,38].

Ploj and colleagues (2000) conducted a similar study and assessed the basal levels of DYN B as well as nociceptin/orphanin FQ (N/OFQ) and met-enkephalin-Arg6 Phe7 (MEAP) in C57BL/6J mice and DBA/2J mice. In addition, the effects of prolonged alcohol self-administration on the level of these peptides were investigated in these mouse lines. They showed that C57BL/6J mice had reduced immunoreactivity (IR) levels of DYN B and MEAP in the NAc, hippocampus, and substantia nigra, decreased DYN B-IR levels in the striatum, and lower MEAP-IR levels in the frontal cortex than DBA/2J mice. C57BL/6J mice, compared to DBA/2J mice, had higher DYN B-IR levels in the pituitary gland and the PAG and higher N/OFQ-IR levels in the frontal cortex and the hippocampus. It was suggested that these strain-related differences between C57BL/6J mice and DBA/2J mice might lead to diverse alcohol-taking behavior [43]. Although these studies showed changes in the level of DYN or KOR between the two mouse lines, further studies are needed to establish a causal relationship between the changes in the DYN/KOR system observed in these studies [32,43] and the difference in alcohol preference/avoidance between the two mouse lines.

Another study observed the impact of repeated episodic ethanol exposure on the tissue contents of endogenous opioid peptides in adolescent Wistar rats.  $\beta$ -endorphin (beta-END) levels in the alcohol-intoxicated state were lower than in the controls; however, this effect was not observed in rats tested three weeks after the last ethanol exposure, showing changes in beta-END levels are due to alcohol administration and does not occur after alcohol administration is ceased for three weeks. A trend toward increased DYN B levels was observed in the pituitary of rats intoxicated with alcohol. At three weeks, there was a higher DYN B level in the substantia nigra of the ethanol-treated group. The effects of ethanol exposure persisted in various brain areas three weeks after exposure to ethanol. In the amygdala, ethanol exposure reduced MEAP levels. In the VTA and substantia nigra, the MEAP levels were higher in ethanol-exposed rats compared to the control group. These changes may play a significant role in the behavioral and molecular actions of alcohol. However, further research is needed to define the exact role of these alterations in different actions of alcohol. For example, whether these changes mediate the rewarding actions of alcohol, its sedative, or other actions.



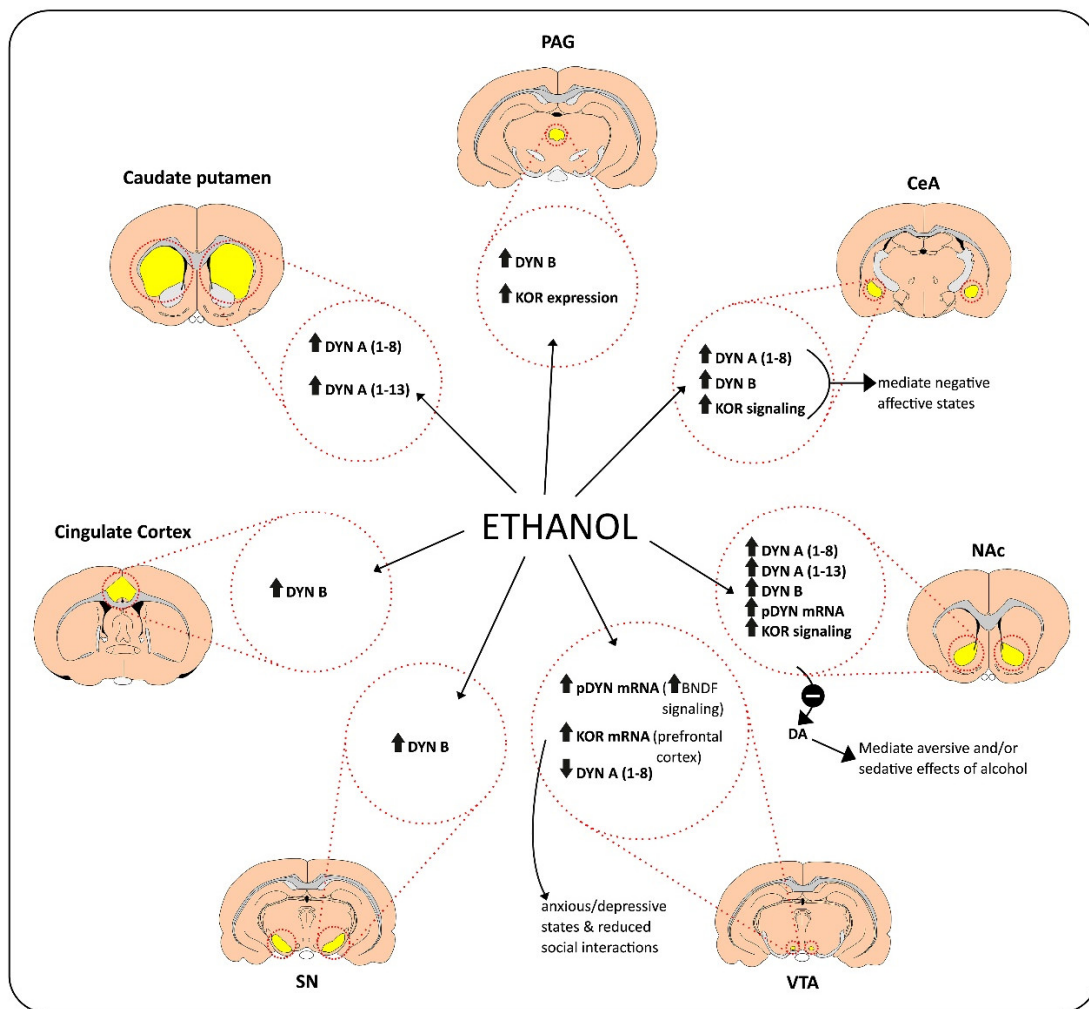
In another study, Nylander's research team measured changes in the level of opioid peptides following a twice-daily injection of alcohol for 13 days in male Sprague-Dawley rats [23]. They discovered that DYN B levels were significantly elevated in the NAc 30 min and 21 days after the last alcohol injection. The peptide level was decreased in the cingulate cortex 30 min following the last dose of alcohol given on day 13. The authors suggested that repeated exposure to ethanol may lead to short- and long-lasting alterations in the turnover of DYN in a site-specific manner [23]. However, further studies are needed to define the relationship between these changes and the effects of alcohol.

Subsequent studies using *in vivo* microdialysis in combination with solid-phase radioimmunoassay were performed to assess the dose-response and time course of the effect of alcohol on the release of opioid peptides, including DYN A 1–8 in different brain regions [33,35,40]. Marinelli and colleagues first measured changes in DYN (1–8) levels in response to different doses of alcohol in the rat NAc shell [40]. Alcohol at a moderate dose (1.6 g/kg, *i.p.*) only transiently (at 30 min) increased the DYN level in the NAc, but a robust rise in the peptide level was observed after the higher dose of alcohol (3.2 g/kg). The authors concluded that the rise in DYN may mediate the aversive effect of alcohol. However, alcohol at both doses also induces sedation, a response observed following KOR activation [50]. Thus, whether the rise in DYN level mediates the aversive effects of alcohol or its sedative effects needs further investigation.

The same laboratory then assessed the effect of alcohol on the level of DYN A (1–8) in the CeA [35]. A microdialysis probe was lowered unilaterally in this brain region and assessed the effect of different doses of ethanol on the level of opioid peptides. While there was no effect of ethanol on the level of met-enkephalin (ME), the level of beta-END and DYN (1–8) was significantly increased following moderate to high doses of ethanol. However, these changes in DYN (1–8) occurred in a delayed manner. Jarjour and colleagues also reported similar delayed changes in the level of DYN (1–8) in the VTA in response to ethanol administration [33]. These authors showed that ethanol had a biphasic effect on the release of beta-END, with low to moderate, but not high, doses of ethanol causing increases in beta-END release in the midbrain/VTA [33]. The ethanol-induced increase in beta-END release in the VTA area of the midbrain is proposed to play a key role in the ethanol-induced stimulation of the mesolimbic dopaminergic neurons and the associated rewarding and reinforcing effects of alcohol [33]. Given that the rise in DYN occurred later than the rise in beta-END, it is unclear what role the rise in DYN A (1–8) would play in this region. Is the rise in DYN level a result of alcohol administration or a compensatory response due to the rise in beta-END and the stimulated dopamine release in the NAc to reduce dopamine back to basal level or even lower?

Bazov and colleagues analyzed post-mortem NAc samples of humans with AUD to assess changes in pDYN and KOR (OPRK1) gene expression and co-expression [26]. No significant differences were observed in pDYN and OPRK1 gene expression levels of post-mortem human NAc samples between those with AUD and controls. A downregulation of dopamine D1 but not D2 receptor expression was seen in individuals with AUDs, suggesting that this dysregulation of dopaminergic pathways may create negative affective states. The expression of D1 and D2 receptors was also strongly correlated with that of pDYN and OPRK1 genes, raising the possibility that there may be co-regulated patterns among these gene clusters [26]. Overall, these findings reveal that alcohol induces changes in the level of DYN and KOR expression in different brain areas (Figure 1).

Logrip and colleagues have shown that alcohol increases ppDYN expression via the brain-derived neurotrophic factor (BDNF). They also found that the blockade of KOR decreases BDNF-induced reduction in ethanol intake [29]. However, the behavioral and molecular changes associated with the peptide release have not been established. Nevertheless, some of these studies show a connection between alterations in the DYN/KOR system and behavioral changes associated with alcohol withdrawal in some brain areas (see Section 3.5).



**Figure 1.** The action of alcohol on the release of dynorphins (DYN) in different brain regions.

### 3.2. The Role of the DYN/KOR System in the Actions of Alcohol

Increasing literature suggests that the DYN/KOR system plays a functional role in the positive and negative reinforcing actions of alcohol, as well as in stress-mediated increases in alcohol consumption in subjects dependent on alcohol (for reviews, see [6–9]). Activation of the DYN/KOR system would be predicted to limit ethanol consumption, whereas pharmacological inhibition of KOR would be expected to increase ethanol consumption. Consistent with this notion, Lindholm and colleagues have shown that U50,488H administration reduced alcohol consumption in rats [39]. Logrip and colleagues examined the effect of U50,488H on alcohol reward using the place conditioning paradigm. They found that the KOR agonist decreases alcohol-induced place preference and the accompanying conditioned locomotor sensitization [30]. These authors concluded that activation of the KOR system decreases alcohol intake due to reduced alcohol reward. However, a decrease in locomotor activity due to KOR activation may be an alternative explanation for the reduced alcohol intake in subjects treated with a KOR agonist, although, in this study, they used lower doses of U50,488H, which did not suppress locomotion. Furthermore, studies have investigated the effects of KOR antagonists on ethanol consumption, and self-administration yielded a mixed result, with both enhancing and suppressing effects of the DYN/KOR system on ethanol intake [41,47]. Nevertheless, the controversy may stem from whether animals had prior alcohol experience and a history of dependence (see Section 3.5).

It is expected that activation of the DYN/KOR system decreases alcohol consumption [41]. However, once the animals are exposed to alcohol chronically, the system is upregulated and may mediate the negative affective states associated with alcohol withdrawal.

In the absence of alcohol, this enhanced DYN/KOR signaling leads to the development of negative affective states associated with increased alcohol consumption. This response can be blocked by the KOR antagonist [47]. For example, nor-BNI, a long-acting KOR antagonist, has been reported to decrease ethanol self-administration in subjects with alcohol dependence while having no effects on non-dependent subjects [48]. However, this result was not recapitulated using a short-acting KOR antagonist, zyklophin. The KOR antagonist, at doses that blocked KOR-mediated actions, did not affect alcohol self-administration in alcohol-vapor-exposed animals compared to air-exposed controls [44]. Together, these data raise concerns about the use of these antagonists (at least the short-acting KOR antagonists) because it may be that sustained inhibition of the DYN/KOR system is needed, as obtained following nor-BNI treatment, to reduce the negative affective states that develop in chronically alcohol-treated subjects to reduce alcohol craving.

### 3.3. Alcohol Self-Administration/Consumption Studies

The DYN/KOR system has been linked to alcohol self-administration. Blednov and colleagues used the two-bottle choice (TBC) paradigm and studied the role of endogenous DYN in alcohol consumption. They discovered that female but not male mice with the deletion of the ppDYN gene consumed significantly lesser alcohol than their wild-type controls [27]. Mice lacking the ppDYN gene also exhibited reduced saccharine consumption, showing that this system may regulate the intake of both alcohol and natural rewarding agents. However, ppDYN knockout and wild-type mice both displayed comparable conditioned taste aversion to alcohol, showing that this system may not mediate the taste-aversive effect of alcohol. Additionally, the deletion of ppDYN did not change the motivational valence of the context when associated with ethanol in the place conditioning paradigm [42]. These knockout mice also did not differ from wild-type mice in signs of acute ethanol-induced withdrawal [27]. However, the rewarding action of ethanol was enhanced in the absence of DYN when mice were tested for state-dependent place preference, i.e., under a drugged state [42]. This effect was recapitulated in the presence of nor-binaltorphimine (nor-BNI), a KOR antagonist [42]. Overall, the latter finding suggests that the endogenous DYN regulates the rewarding action of alcohol but not the motivational valence of context [42]. This enhanced state-dependent CPP may also explain why knockout mice consume less alcohol than wild-type mice, i.e., animals may find alcohol too rewarding and thus reduce their intake.

Another study used KOR knockout and their wild-type controls in the TBC paradigm to examine the role of the DYN/KOR system in oral alcohol self-administration. Mice with the targeted disruption of KOR showed a decreased preference for alcohol and saccharin but an increased preference for quinine [38]. These authors suggested that the disruption of the DYN/KOR system affects orosensory reward through central mechanisms, which, in turn, reduces alcohol palatability [38]. Interestingly, KOR knockout mice displayed enhanced evoked-dopamine levels in the NAc in response to alcohol administration [49]. The ability of the KOR blockade to regulate the ethanol-evoked dopamine release in the NAc led to the conclusion that a decrease in the activity of the DYN/KOR system might enhance the reinforcing effect of ethanol [49]. Thus, it may be that the rewarding action of alcohol is enhanced in knockout mice, which may experience reward at a lesser volume of alcohol than wild-type mice. An alternative explanation is enhanced taste aversion in null mice. However, as stated above, Blednov and colleagues found no difference in alcohol taste aversion between mice of the two genotypes [27].

One study proposed that the DYN/KOR system is activated following chronic alcohol administration, and the increased activity of this system following withdrawal results in increased alcohol self-administration. Therefore, KOR antagonists should reduce the negative affective states associated with alcohol withdrawal [36]. Additional evidence shows that DYN plays a functional role in the transition to alcohol dependence and not in the acute reinforcing effects of alcohol [36]. The authors concluded that KOR is a mediator of the negative affective states associated with alcohol withdrawal and repeated stress and

plays an important role in subjects dependent on alcohol. Thus, the DYN/KOR system may be a potential target for treating AUD in subjects undergoing abstinence, particularly those experiencing stress in their lives [48].

### 3.4. *Kappa Opioid Alterations in Anxious and Depressive States*

The DYN/KOR system plays a part in regulating negative affective states due to alcohol withdrawal (for a review, see [48]). Various models are available to investigate anxiety- and depressive-like behavior following alcohol administration and withdrawal in rodents. Many studies sought to observe how alcohol consumption induces or relates to anxiety or depression. One study used a light–dark box (LDB), shelter-seeking and risk-taking behaviors in the concentric square field test, and locomotion in the open field to assess the impact of prenatal ethanol exposure (PEE) on anxiety- and depression-like behaviors later in life. They also investigated whether this treatment would induce any DYN or KOR gene expression changes later in life. PEE-induced alterations in gene transcription were associated with an anxiety-prone phenotype, as rats showed increased avoidance of the undesirable areas of the LDB and concentric square field, increased shelter-seeking in the concentric square field, and a decrease in exploration in all behavioral tasks. Additionally, it was found that animals exposed to ethanol prenatally had a selective upregulation of pDYN mRNA in the VTA and KOR mRNA in the prefrontal cortex. This study provides evidence suggesting that the PEE-induced anxiety-prone phenotype correlates with alterations in DYN and KOR gene expression [46]. However, further studies are warranted to confirm the causal relationship of such gene expression alterations to behavioral changes.

Another study aimed to determine the role of the DYN/KOR system in regulating anxious behavior during acute withdrawal from ethanol [12]. An elevated plus maze (EPM) was used to record anxious behaviors during ethanol withdrawal. nor-BNI was administered to some rats to assess how KOR blockade affects ethanol self-administration under this condition. Results showed that chronic ethanol liquid diet exposure caused an increase in anxiety-like behaviors, as evidenced by a reduction in open-arm exploration. Additionally, the rats experiencing ethanol withdrawal had a significant decrease in the number of total arm entries. Anxiety-like behaviors associated with ethanol withdrawal were blocked by the KOR antagonist. These results suggest that the DYN/KOR system may mediate the anxiety-like behaviors associated with alcohol withdrawal [12].

The involvement of the DYN/KOR system in anxiety-related behaviors during prolonged ethanol abstinence was also explored. These authors used the EPM to investigate the effect of nor-BNI on anxiety-like behaviors induced by a mild stressor in abstinent rats. Stress exposure decreased the amount of time that alcohol-withdrawn animals spent in the open arms, showing signs of anxiety-like behaviors in these animals compared to their controls. The KOR antagonist reduced these behaviors, indicating that the DYN/KOR system is a critical mediator of stress-induced anxiety associated with alcohol withdrawal and that while KOR antagonism may be sufficient to reduce the heightened stress response found after long-term ethanol withdrawal, direct activation of the DYN/KOR system may not be required to elicit stress-related behaviors [10].

The forced swim test (FST) is used to study depression-like behaviors in rodents [51]. Sperling and colleagues showed that nor-BNI lowers the enhanced ethanol intake caused by forced-swim stress, whereas the KOR agonist U50,488 increases ethanol preference and ethanol drinking in mice [45], implying that similar processes are involved in the development of ethanol withdrawal- and KOR agonist-induced behavioral alterations [12].

FST was also used to examine the ability of nor-BNI to reverse depressive-like behavior in rats chronically exposed to ethanol. Results showed that rats chronically exposed to ethanol exhibited increased immobility time in the FST during acute ethanol withdrawal and protracted ethanol abstinence, which were both reversed by nor-BNI, suggesting that the DYN/KOR system mediates depressive-like states linked to alcohol withdrawal [34]. Overall, these findings suggest that the DYN/KOR system mediates the negative affective states associated with alcohol withdrawal.



### 3.5. Neuroanatomical Sites of Actions of the DYN/KOR System in Regulating Alcohol Consumption and Affective Signs of Ethanol Withdrawal

Different brain regions have been implicated in the regulatory actions of the DYN/KOR system on alcohol consumption. Likewise, as described above, alcohol induces alterations in the DYN/KOR system in different brain regions. Several techniques have been used to investigate the role of the DYN/KOR system in these brain regions in response to alcohol consumption and withdrawal. The CeA expresses a high level of DYN and KOR [25]. This brain region has also been associated with binge drinking and alcohol intake linked to dependency [31]. One study found that binge-like alcohol consumption is mediated, at least in part, by DYN/KOR signaling in the CeA. Acute alcohol administration causes the release of DYN in the CeA [35], and persistent alcohol vapor exposure increases DYN peptide expression as well as KOR signaling in the CeA [37]. In this study, one experiment looked into the effect of intra-CeA injections of a KOR antagonist on binge-like alcohol consumption using the dinking in the dark approach. Mice were given microinjections of nor-BNI into the CeA, which reduced alcohol consumption. They also used a chemogenetic approach to deactivate DYN-containing neurons to recapitulate the results obtained with nor-BNI. Adult male pDYN-IRES-Cre mice were sacrificed for in situ hybridization or injected with a virus harboring Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) that targeted the CeA. The membrane potential of DREADD-expressing neurons was significantly reduced after a bath injection of clozapine-*N*-oxide. Thus, these findings demonstrate that direct inhibition of KOR in the CeA reduces alcohol consumption, implying that KOR signaling within the CeA plays a functional role in regulating binge-like alcohol intake [25].

Another study investigated the role of KORs in the extended amygdala in alcohol dependence. Alterations in the expression of DYN or KOR caused by chronic alcohol consumption can affect areas such as the NAc shell and the CeA. Previous studies have suggested that KOR may mediate the negative reinforcing actions of alcohol consumption rather than its positive reinforcing effects. Thus, negative affective states associated with alcohol withdrawal are hypothesized to make alcohol a negative reinforcer. KOR antagonists decrease the negative affective state associated with alcohol withdrawal. These results suggest that a KOR antagonist may enable the subjects to stay sober for a longer time and make abstinent individuals seek therapy and compel them to stay on medications for the management of their AUD [47].

Studies by Haun and colleagues (2020) have examined the role of the KOR system in the BNST in binge drinking in male and female mice. They also measured the impact of a KOR antagonist on sucrose intake. Females consumed more alcohol and sucrose during the first two hours than male mice. Bilateral intra-BNST injections of nor-BNI decreased binge drinking and blood alcohol levels in both male and female mice and also reduced sucrose intake, but to a lesser extent than alcohol consumption. Locomotor activity was unaffected by nor-BNI. U50,488 was also administered systemically in mice and resulted in a large increase in alcohol consumption. When nor-BNI was administered in the BNST after systemic U50,488 injection, nor-BNI blocked U50,488-induced increases in alcohol intake and blood alcohol levels. The authors concluded that the DYN/KOR system in the BNST plays a role in binge drinking in both male and female mice [28].

### 3.6. Impacts of Gender/Sex

Past research has shown a significant difference in alcohol consumption between the sexes in both biological and social contexts [52]. Women have been shown to drink less in a social setting where they are excluded than men [53]. Interestingly, women are more likely to be lifetime abstainers and less likely to consume alcohol, engage in problem drinking, develop AUDs, and experience withdrawal after a drinking episode compared to men [54]. Although there is a large body of evidence exploring the bidirectional relationship between sex and alcohol consumption, there is little research regarding the role of DYN/KOR in these mechanisms in humans. A previous study reported a higher level of available KOR

in healthy males than females [55]. This appears to change in subjects with AUDs, in which there is reduced KOR availability in the amygdala and pallidum of males with AUDs than in healthy controls. This difference was only significant in the amygdala between females with AUDs and their healthy controls [56]. Interestingly, KOR upregulation has been suggested to enhance the reward-related effects of addictive drugs in males only (for a review, see [57]). On the other hand, KOR availability was reduced in subjects with decreased craving and alcohol intake following naltrexone treatment [58], as naltrexone may cause the release of endogenous DYN to reduce drinking and craving and downregulate KORs. Future studies are needed to test this possibility.

Preclinical data show that KOR-induced antinociception but not aversion is attenuated in female C57BL/6N mice rather than male mice [59]. Differences in the consumption of alcohol may also depend on sex in rodents. Evidence suggests that males and females respond differently to KOR manipulation in drug-related behaviors (for a review, see [57]). The sex-related differences in the action of nor-BNI to regulate ethanol consumption that have been observed in adults may be due to females being more stressed and anxious when housed individually. This may have caused the females to consume less ethanol following the KOR blockade because nor-BNI successfully relieved their anxiety, so ethanol was no longer needed to relieve their anxiety.

Adult males and females exhibited higher blood ethanol concentrations than adolescent rats, possibly due to younger rats metabolizing ethanol faster than adults when administered a small dose of ethanol. Male and female adolescent mice showed greater locomotor activity than their adult counterparts, and female adults exhibited greater locomotor activity than male adults, which could impact alcohol intake [60]. As stated above, female but not male mice lacking the ppDYN gene consumed reduced amounts of alcohol compared to their wild-type counterparts [27]. Overall, these observations suggest that there may be male/female differences in alcohol consumption mediated by the DYN/KOR receptor system or, at least, that adult males and females respond differently to KOR ligands to alter alcohol consumption.

### 3.7. Impacts of Age

Age-related differences in alcohol consumption are well documented [61,62]. More recent data show that 39.7% of 12- to 20-year-olds have admitted to having at least one drink in their lifetimes, showing how, as time progresses, the consumption of alcohol in the young decreases. Moreover, elevated levels of ethanol at this age can be problematic as early ethanol use and abuse is a strong predictor of lifetime alcohol abuse and dependence [63]. With regards to receptor availability, it was found that KOR availability in the amygdala and pallidum is reduced in males with AUDs than in healthy controls. Still, there was no effect of age in this regard [56]. Considering that there are limited human studies assessing the impact of age concerning the role of the DYN/KOR system in AUDs, further studies are needed to determine the role of endogenous and exogenous DYN and other KOR ligands in different age groups with AUDs.

Preclinical data suggest that adolescents may be less sensitive to the aversive properties of drugs of abuse than adults, and they also appear more sensitive to the rewarding actions of addictive drugs [64–66]. The DYN/KOR receptor system may be important in this age-related difference in alcohol consumption [60]. There was an age-related difference in alcohol consumption where adolescent males consumed more ethanol than adults. However, adolescent males had a significantly lower blood ethanol concentration than adults. The ability of the KOR antagonist to impact ethanol consumption varied based on age, as nor-BNI did not alter alcohol consumption in adolescents of either sex. On the other hand, nor-BNI increased alcohol intake in male adults and decreased ethanol consumption in female adults. These results suggest that the DYN/KOR system regulates alcohol intake differently between adolescents and adults as well as in male and female adults [60].

#### 4. Conclusions and Application

The DYN/KOR system plays a functional role in regulating the negative symptoms of alcohol withdrawal. This system is involved in regulating the depressive-like states associated with alcohol dependence. Moreover, KORs have been seen to regulate stress-mediated anxiety and the anxiety caused by alcohol withdrawal. KOR antagonists can help decrease increased alcoholism in both long-term and short-term addictive states. Moreover, this system is key in alleviating the negative affective states in both adolescents and adults with AUDs. The studies above show how targeting KORs directly impacts the symptoms of alcohol withdrawal. The CeA and BNST may be prominent brain areas where DYN exerts its regulatory action on the negative affective states associated with alcohol withdrawal.

KOR agonists and antagonists have been successful in reducing the intake of alcohol in animals and possibly in humans. However, KOR agonists devoid of aversive effects need to be developed to promote patient compliance. On the other hand, a KOR antagonist might be a better option to reduce or prevent negative affective states associated with alcohol withdrawal. However, one should be cautious about the promise of KOR antagonists, given that some of these ligands have failed to provide efficacy in clinical trials [67,68]. Additionally, some of these antagonists have unusual pharmacokinetics and pharmacodynamics when administered to rodents, such as JTDic, GNTI, and nr-BNI. They have a slow onset of action but a long-lasting effect with poor solubility [69]. Thus, novel ligands that only show selectivity toward KORs and act faster are preferable. One such compound is LY2456302, which displays selectivity for KORs [70]. This KOR antagonist also had good safety and tolerability when injected with ethanol [71] in cocaine-dependent subjects and healthy volunteers [72].

Interestingly, this KOR antagonist was efficacious in decreasing alcohol consumption and motivation to self-administer alcohol. It also attenuated the reinstatement of alcohol-seeking behavior in rats [70,73]. Another KOR antagonist, CERC-501, also shows some efficacy in a preclinical model of alcoholism, but it has a half-life of 40 h, which may not be ideal clinically [69]. However, some of these KOR antagonists failed to show efficacy in altering drug use metrics in nicotine- or cocaine-dependent individuals (for a review, see [74]). Therefore, more research is needed to show the efficacy and safety of these ligands in subjects with AUDs or addictive drug dependence.

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