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REVIEW

The dynorphin/κ-opioid receptor system and its role in psychiatric disorders

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Abstract The dynorphin/κ-opioid receptor system has been implicated in the pathogenesis and pathophysiology of several psychiatric disorders. In the present review, we present evidence indicating a key role for this system in modulating neurotransmission in brain circuits that subserve mood, motivation, and cognitive function. We overview the pharmacology, signaling, post-translational, post-transcriptional, transcriptional, epigenetic and *cis* regulation of the dynorphin/κ-opioid receptor system, and critically review functional neuroanatomical, neurochemical, and pharmacological evidence, suggesting that alterations in this system may contribute to affective disorders, drug addiction, and schizophrenia. We also overview the dynorphin/κ-opioid receptor system in the genetics of psychiatric disorders and discuss implications of the reviewed material for therapeutics development.

Keywords Dynorphin · κ-Opioid receptor · Psychiatric disorder · Pharmacology · Neuroanatomy

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Introduction

Psychiatric disorders constitute a global health problem. Depression, drug addiction, and schizophrenia affect ca. 120, 90, and 25 million people, respectively [1]. These numbers, however, do not accurately reflect the disease burden attributable to psychiatric disorders as one out of four families have at least one affected member. Although phenotypes differ between and within these disorders, they are characterized by alterations in cognition, emotion, motivation, and stress reactivity. Co-morbidity between disorders is high suggesting common neural substrates [2]. Consistently, imaging studies have shown altered activity in the amygdala, hippocampus, basal ganglia, and prefrontal cortex of psychiatric patients [3]; areas involved in stress responsiveness, emotional reactivity, goal-directed behavior, motivation, and executive function.

The κ-opioid receptor (KOR) and its postulated endogenous ligands, the dynorphin peptides (DYNs) [4], are enriched in the above brain regions and modulate neurotransmission therein. Increasing data suggest that dysregulation of this system may contribute to the development and maintenance of various psychiatric disorders [5], and recent years have seen an explosion of studies on this topic. In the present review, we first overview the pharmacology, signaling, post-translational, post-transcriptional, transcriptional, epigenetic, and *cis* regulation of the DYN/KOR system. We then review functional neuroanatomical, neurochemical and pharmacological evidence supporting a physiological role for this system in modulating neurotransmission in brain regions implicated in the pathogenesis and pathophysiology of drug addiction, affective disorders, and schizophrenia. Lastly, we overview the DYN/KOR system in the genetics of psychiatric disorders and discuss

implications of the reviewed material for medications development.

DYN/KOR system—pharmacology, signaling, and post-translational regulation

DYN peptides are derived from prodynorphin (PDYN) [6]. Although DYN A1–17 (DYN A) is considered the endogenous KOR ligand [7], a number of smaller, biologically active DYN as have been identified including DYN A1–8 and DYN A1–13 [8, 9]. Other biologically active DYNs include DYN B1–13 (DYN B/rimorphin), DYN B1–29 (leumorphin), DYN A/B1–32 (big DYN) and α- and β-neoendorphin [10–14]. The affinity and efficacy of these peptides differ with DYN A being the most potent and β-neoendorphin the least [15, 16]. Importantly, DYNs bind with high affinity to other opioid and non-opioid receptors (e.g., acid-sensing ion channels and NMDA receptors), [17–19]. The CNS distribution of DYNs varies with species and brain region. In general, α-neoendorphin is the most abundant DYN and DYN A the least with the largest expression difference observed in substantia nigra pars compacta (SNc) [20–22].

Although additional of processing enzymes have been identified, DYN biosynthesis in vivo in the mouse brain appears governed primarily by the non-selective proteases cathepsin L, PC1, 2 and 3 and CPE [23–25]. Their regional expression and functional coupling them between may therefore underlie regional variations in DYN expression [26, 27]. Interestingly, cathepsin L and α-neoendorphin and DYNs A and B display vesicular co-localization in mouse primary cortical neurons [25], while PDYN is observed in vesicles in axonal and synaptic compartments in the rat brain [28]. Depolarization-induced PDYN processing and release of DYN A and B have been observed in rat primary cortical neurons. More work, however, is needed to identify which DYNs are released in vivo; clues to which may come from investigation of the roles of post-translational modifications and product inhibition in DYN biosynthesis [26, 29]. This information may help explain the relative abundance of α-neoendorphin in the brain [20–22], despite it being the least resistant to proteolytic cleavage in vitro [30].

KOR is a G-protein coupled receptor [31–33]. At least three subtypes have been suggested based on pharmacology. It seems likely, however, that these result from alternative splicing, post-translational modifications and/or protein–protein interactions [34, 35]. KOR reportedly couples to both inhibitory $G_{\beta\gamma}$, $G\alpha_i$, $G\alpha_o$, $G\alpha_z$ and $G\alpha_{16}$, and stimulatory, $G\alpha_s$, G-proteins [36]. Nanomolar ligand concentrations result in coupling of KOR to inhibitory G proteins, decreasing membrane excitability and transmitter release via stimulation of K^+-channel activity (e.g., in guinea pig substantia gelatinosa slices) [37], and inhibition of Ca^{2+}-channel and presynaptic release machinery activity (e.g., in rat primary nodose root ganglion neurons [38], and the paraventricular nucleus of the hypothalamus [39], respectively). In contrast, sub-nanomolar ligand concentrations may result in coupling of KOR to $G\alpha_s$ and produce opposite effects on ion channel conductance, prolonging action potential duration [40]. Not surprisingly therefore, KOR agonists produce an inverted U-shaped dose-response curve. The complexity of KOR signaling is reflected in the ability of agonists to stimulate, inhibit and/or not affect all major second messenger systems (i.e., cAMP, IP_3/DAG, and Ca^{2+}) depending on cell line/type and experimental conditions used, and in the numerous downstream effectors identified [36, 41, 42].

Post-translational regulation of KOR agonist responsiveness occurs through three distinct processes: (1) desensitization (sec–min), (2) internalization (min–h), and (3) resensitization or down-regulation (h–days) [43]. DYNs A and B, α-neoendorphin, the naturally occurring KOR agonist, salvinorin A, and several synthetic agonists (e.g., U50,488H and U69,593) induce desensitization, internalization and down-regulation of KOR in heterologous expression systems [16, 44–46]. In contrast, other synthetic agonists and antagonists (e.g., etorphine and norbinaltorphimine (BNI), respectively) have opposite effects. These paradoxical findings may be explained by chaperone-like effects of some membrane-permeant ligands as suggested by their varying ability to also promote resensitization [47, 48]. Ligands may also direct KOR signaling via differential activation of downstream effectors [49]. The notion that regulation of KOR signaling and trafficking/biosynthesis is ligand-directed has spawned interest in the development of drugs locking this receptor in favorable conformational states such that dissociation of desired and undesired behavioral responses to its activation will be maximal.

Regulation of *pdyn* and *oprk1* in *cis* (gene regulation intrinsic to DNA)

No consensus gene and protein nomenclature exist across species. In accordance with the human gene nomenclature [50], we denote abbreviated gene names in italic and capital letters and abbreviated protein names in capital letters. For other species, abbreviated gene and protein names are preceded by species when appropriate and denoted in italic and lowercase letters and capital letters, respectively. The same nomenclature is applied when referring to abbreviated gene and protein names in general. We hope that these distinctions will aid interpretation of the reviewed material. *pdyn* has four exons in human,

mouse and rat [51–53]. *PDYN* contains multiple transcription start sites located in exons 1 and 4 and introns 1 and 2 [54, 55]. Although in silico analysis suggests a similar transcription start site usage profile for mouse *pdyn*, putative human-specific alternative promoter usage has been shown/predicted for brain and testis with transcription start sites in exon 4 and intron 2, respectively. Consistently, brain- and testis-specific *PDYN* transcripts have been identified [54, 56, 57]. Like *pdyn*, the KOR gene, *oprk1*, has four exons in human, mouse and rat [58–60]. Mouse *oprk1* reportedly has dual promoters and multiple transcription start sites located in exon 1 and intron 1 [61]. Although in silico analysis suggests a similar transcription start site usage profile for *OPRK1*, human-specific alternative promoter usage is predicted for brain and testis with transcription start sites in exon 2 and intron 2, respectively [55]. As transcription start sites are coupled to *cis*-regulatory elements (DNA sequences that influence gene expression) [62], further characterization of the promoters of *PDYN* and *OPRK1* may provide insights into transcriptional control of these genes and their dysregulation in psychiatric disorders.

PDYN is regulated in *cis* by copy number variants and single nucleotide polymorphisms (SNPs) located in the promoter region of this gene (Fig. 1) [63–65]. Among these, attempts have been made to associate rs35286281 (copy number variant) (unsuccessful) and rs1997794 (SNP) (successful) with have been associated with allelic imbalance (differential expression of alleles at one or more loci) in the cortex and cerebellum [52, 64, 66, 67]. Support for a role of these variants in *PDYN* regulation comes from in silico analysis and in vitro binding studies suggesting that they may be targeted by the transcriptional control protein, AP-1 [68, 69]. Alternatively, the causative variant may be one in linkage disequilibrium (nonrandom association of alleles at two or more loci) with rs35286281 and rs1997794. A candidate variant is rs910080 (SNP) located in the 3′-untranslated region of *PDYN* [64]. Interestingly, in the one subject analyzed who was homozygous for this locus, no allelic imbalance was observed [69]. Moreover, rs910080 overlaps with a response element bound by the transcriptional control protein REST in vivo in various cell lines (Fig. 1) [70], and dominant negative REST increases endogenous *PDYN* expression in human neuroblastoma SH-SY5Y cells [71]. Consistent with roles of 3′-untranslated region variants in *PDYN* regulation, a region comprising *PDYN*, the neighboring gene *STK-35* and regions 3′ of the two bears the signature of recent positive selection (evolutionary increase in the frequency of a genomic region) [72]. Although rs35566036 (indel) located in the promoter of *OPRK1* has been shown to influence reporter gene expression in vitro (Fig. 1) [73], comparatively little is known about regulation of this gene in *cis*. Further assessment of the mechanisms by which rs35286281, rs1997794, rs910080, and rs35566036 affect gene expression is important as they have all been associated with psychiatric disorders (overviewed below).

Epigenetic regulation of *pdyn* and *oprk1*

Repeated cocaine administration increases *pdyn* expression in the striatum of rodents and non-human primates (reviewed below). Cocaine was recently shown to increase active histone marks (i.e., H4 acetylation) without affecting repressive ones (i.e., H3K9 and H3K27 methylation) on a nucleosome spanning the transcription start site in mouse *pdyn* [74]. Methylated CpG islands (genomic regions containing a high frequency of CpG dinucleotides) and dinucleotides have been identified in the promoters of *PDYN* and *OPRK1* and in intron 2 of *OPRK1*, but the role of this epigenetic mark in the transcriptional control of these genes is unclear [75–77]. Mouse *oprk1* expression is affected by trophic agents [78]. Four-day treatment of mouse P19 cells with retinoic acid results in repression of *oprk1* expression via recruitment of chromatin remodeling factors (i.e., BAF155 and BRG-1) to promoter 1, acquisition of repressive histone marks (i.e., H4 deacetylation and H3K9 methylation), subsequent chromatin condensation by two adjacent nucleosomes spanning the transcription start site and parallel alterations in transcriptional control protein binding. If further differentiated with NGF, promoter 2 is activated via transcriptional control protein recruitment and acquisition of active histone marks (i.e., H3K9 demethylation and H3K4 dimethylation). It should be noted that while some predictions can be made on the basis of a 'histone code' [79], the presence of active or repressive chromatin marks on a promoter does not always correlate with gene expression [80]. The predictive value of a single mark or even a combination of marks on a given transcriptional outcome is therefore limited. Moreover, histone modifications and DNA methylation are not all classical epigenetic marks (i.e., heritable changes in chromatin function without alterations in the primary DNA sequence). Thus, assessment of their heritability may aid in the evaluation of *PDYN* and *OPRK1* as susceptibility loci for psychiatric disorders.

A gene encoding a long non-coding RNA, *AK090681*, is transcribed from the opposite strand of *PDYN* [81]. Long non-coding RNAs may regulate gene expression via chromatin remodeling [82]. However, *PDYN* and *AK090681* may be separate but overlapping transcription units as suggested by the exon locations of *AK090681*. Moreover, this gene appears to be actively transcribed in human embryonic stem cells while *PDYN* does not. Interestingly, the promoter of *AK090681* contains a CpG island which methylation status may correlate with that of

Fig. 1 To scale schematics of *PDYN* (**a**) and *OPRK1* (**b**). Shown are functional sequence variants associated with psychiatric disorders (e.g., rs35286281) and transcriptional control proteins implicated in the regulation of these genes (positioned relative to the transcription start sites). Transcriptional control proteins for which there is in vivo evidence of binding are depicted above the sequence (e.g., REST), and those for which only in vitro evidence is available below the sequence (e.g., AP-1). ‖ genomic regions omitted for clarity, *boxes* exons, *white boxes* coding regions, *black arrows* transcription start sites, *green arrows* alternative transcription start sites

H3K27, suggesting that *AK*090681 may be involved in human embryonic stem cell differentiation [75, 76]. Moreover, rs6136489 (SNP) located in the promoter of *AK090681* was recently associated with mean platelet volume (parameter affecting for example cardiovascular function) in a genome-wide meta-analysis [83]. Two transcripts lacking exons 3 and 4 have been reported for *AK090681* [55, 84]. These exons enclose *PDYN*, and exon 3 and intron 3 of *AK090681* contain response elements bound by the 'master weaver' CTCF and cohesin in vivo in a number of cell lines [85, 86]. CTCF may be a heritable component in epigenetic control, which regulates the interplay between DNA methylation, higher-order chromatin structure and lineage-specific gene expression [87]. Thus, the transcription units of *AK090681* and *PDYN* may be separated by CTCF-mediated chromatin looping.

Regulation of *pdyn* and *oprk1* in *trans* (gene regulation extrinsic to DNA)

Although in vitro studies have implicated numerous transcriptional control proteins in *PDYN* regulation (Fig. 1) [88, 89], we will limit our discussion to those for which there are evidence of binding in vivo. *PDYN* is targeted by the glucocorticoid receptor (GR) in human A549 cells (Fig. 1) [90]. GR binding is responsive to the synthetic GR agonist, dexamethasone, whereas *PDYN* expression is not. Thus, GR may either not be involved in *PDYN* regulation, or loss or gain of interactions in *cis* and/or *trans* are required to unmask an effect of glucocorticoid signaling on *PDYN* expression. Although the cognate response element(s) has not been identified, support for the latter notion comes from studies showing that treatment of rats with dexamethasone or another synthetic glucocorticoid, budesonide, alters hippocampal and spinal cord *pdyn* expression, respectively [91–93]. Moreover, GR binding to *PDYN* in A549 cells does not correlate with that of RNA polymerase II, suggesting that *PDYN* is not actively transcribed in these cells [90]. Although a useful indicator, binding therefore does not exclude/include involvement of a transcriptional control protein in the regulation of a given gene [94]. Moreover, whether the native chromatin state is present in immortalized cell lines/cancers has been questioned. Direct involvement of GR in *PDYN* regulation may be of clinical relevance given the growing body of evidence suggesting a role for the DYN/KOR system in stress (reviewed below).

The transcriptional control proteins AP-1, CREB and DREAM are implicated in a variety of physiological processes including Ca^{2+}-signaling and synaptic plasticity [95, 96], and appear to play overlapping roles in *pdyn* regulation [97–100]. AP-1 and CREB may activate, repress, or not affect *pdyn* expression depending on interactions in *cis* and *trans* [101–104]. It should be noted, however, that with the exception of a non-canonical AP-1 site identified by Naranjo et al. [68], neither of the response elements for AP-1 and CREB identified in mouse and rat *pdyn* is present in *PDYN*. Moreover, *PDYN* has never been identified as a target for any AP-1 or CREB family member in vivo [105–108]. Thus, identification of the response elements in *pdyn* bound by AP-1 and CREB in vivo may provide valuable insights into species- and/or allele-specific regulation of this gene and its dysregulation in psychiatric disorders. Further insights into the roles of these transcriptional control proteins in *PDYN* regulation may also come from protein interaction studies as the CREB family member αCREM has been implicated transcriptional control of this gene via interaction with DREAM [99]. Contrary to AP-1 and CREB, the expression data on DREAM is consistent and suggest that this protein represses *pdyn* expression [98, 109]. Interestingly, the aversive effects of Δ-tetrahydrocannabinol, but not cocaine and morphine, are potentiated in a KOR-dependent manner in *kcnip3* knockout mice, implicating this transcriptional control protein in drug-induced dysregulation of the DYN/KOR system [110].

The transcriptional control proteins AP-2, C-MYC, Ikaros, MAD and MAX are involved in cell proliferation, lineage commitment and differentiation [111–113], and have been implicated in the transcriptional control of mouse

oprk1 [78]. Ikaros suppresses *oprk1* expression in P19 cells, while a shift in binding of C-MYC and MAX to MAD and MAX parallels the transition from high to low constitutive expression of *oprk1* transcripts initiated from promoter 1 which occurs during development. AP-2β appears to activate transcription from promoter 2, which is higher postnatally. Interestingly, AP-2β is implicated in the regulation of a number of genes involved in monoamine neurotransmission [e.g., *SLC6A3* (dopamine transporter (DAT)] and *TH* [tyrosine hydroxylase)] and rs55733871 (copy number variant) located in intron 2 of the AP-2β gene, *TFAP2B*, is associated with anxiety-related personality traits [114]. Thus, gene–gene interaction studies on *OPRK1* and *TFAP2B* are warranted. It should be noted, however, that of the cognate response elements for these transcriptional control proteins identified in mouse, only two are present in *OPRK1* [60]. Moreover, *OPRK1* appears not to be targeted by C-MYC in vivo [115, 116]. These findings support a role for *cis*-regulatory divergence (measure of the evolutionary conservation of genomic regulatory regions) in *pdyn* and *oprk1* regulation as has been suggested for human and mouse on a genome-wide scale [117].

REST is implicated in development and disease (e.g., Huntington's disease) [118]. Of the two response elements for REST identified in *PDYN* (Fig. 1) [70], binding to the one mentioned above (see Regulation of *pdyn* and *oprk1* in *cis*) is not apparent in all cell lines analyzed indicating a role for REST in lineage-specific regulation of *PDYN* [119]. Although the cognate response element(s) has not been identified, *OPRK1* may also be restrictively targeted by REST in vivo in human glioblastoma U87 cells [70, 120]. The transcriptional control protein MeCP2 has been implicated in development and disease (e.g., Rett syndrome) [121], and was recently found to target mouse *oprk1* and activate its transcription in vivo in the hypothalamus perhaps via recruitment of CREB [121]. Interestingly, REST, MeCP2 and CREB are parts of a regulatory network identified using comparative genomics involving multiple microRNAs (e.g., miR-9 and miR-132/212) [123–125]. Intriguingly, like dominant negative REST, ectopic expression of miR-9 increases endogenous *PDYN* expression in SH-SY5Y cells [71]. Moreover, parts of this network have recently been implicated in psychiatric disorders [126–129]. Thus, aberrant network activity may underlie some instances of altered *PDYN* and *OPRK1* expression in these disorders.

Post-transcriptional regulation of *pdyn* and *oprk1*

Multiple *pdyn* transcripts have been reported for human, mouse and rat [54–57, 130], and alternatively spliced *pdyn* mRNAs have been reported [54, 57]. Transcript multiplicity has been suggested to provide yet another level of control over PDYN and KOR expression in addition to those overviewed above [131]. In some cases, however, the translation products of these messages may have novel functions as suggested by the nuclear localization of the truncated PDYN 'T1' in African green monkey COS-1 cells and their sequence similarity to transcriptional control proteins [54, 132]. Multiple *oprk1* transcripts have been reported for human, mouse, and rat [55, 59, 61]. Although alternative splicing has been reported for human *OPRK1* [133], post-transcriptional regulation of *oprk1* has been studied almost exclusively in mouse. Differential stability and translation efficacy has been reported for *oprk1* transcripts in P19 cells [134, 135]. EGF stimulation results in SHP-2-mediated dephosphorylation of the growth factor receptor bound protein GRB7, recruitment of the HUR–exportin-1 complex and nuclear export of *oprk1* mRNA in P19 cells and dorsal root ganglion neurons [136]. EGF and the axonal guidance cue netrin-1 also induce FAK-mediated phosphorylation of GRB7 and derepression of *oprk1* mRNA translation in the cytoplasm [137–139]. Intriguingly, depolarization-induced, possibly COPB1 and HUR-mediated, axonal transport and local translation of mouse *oprk1* transcripts in terminals have been demonstrated in these cell models [140–142]. The high spatial and temporal resolution analyses of mouse *oprk1* epigenetic, transcriptional and post-transcriptional regulation provided by Wei Laboratories, Inc., is key to defining the physiological role of the DYN/KOR system [143], and parallel studies in other species are warranted.

Functional DYN/KOR neuroanatomy

The CNS distribution of DYN and KOR has been detailed elsewhere [144]. Therefore, this review will focus on their localization and function in brain circuitry implicated in psychiatric disorders. Moderate to high DYN and KOR expression is observed in the cortex, basal ganglia, hippocampus, amygdala, thalamus, as well as monoaminergic midbrain and brainstem structures. In some regions, clear overlap of DYN and KOR expression is not observed, suggesting that signaling therein may depend on volume transmission. Alternatively, distribution discrepancies may be explained by differences in methodology (untreated vs. colchicine-treated), species, or gender.

Dorsal and ventral striatum

The dorsal and ventral striatum, which include the nucleus accumbens (NAcc) and olfactory tubercles, are involved in movement execution/habit formation and motivation/reward, respectively. Both the dorsal and ventral striatum are primarily composed of medium-sized spiny neurons

(MSNs) that receive convergent glutamatergic and dopaminergic (DAergic) inputs [145]. The ventral striatum receives dense DAergic fibers from the ventral tegmental area (VTA) and to a lesser extent from the SNc. Conversely, the dorsal striatum is primarily innervated by SNc-originating DA fibers, with little VTA innervation. KORs and DYN are highly enriched in dorsal and ventral striatal compartments (see Fig. 2) [144, 146–150]. In both, DYN is expressed in GABAergic MSNs that preferentially express D1 DA receptors [151, 152]. Electron microscopy studies have shown that striatal KORs are localized on DA varicosities in close apposition to the dopamine transporter (DAT) and, to a lesser extent, on asymmetric, presumably excitatory synapses [149, 153]. Systemic or local administration of KOR agonists into either striatal sub-region decreases basal and stimulated DA efflux [154–157]. Conversely, systemic administration of the selective KOR antagonist, norbinaltorphimine (nor-BNI) elevates NAcc dialysate DA levels [158]. Furthermore, striatal and accumbal KORs tonically inhibit basal dopamine overflow [155, 159] such that intra-striatal or intra-accumbal KOR blockade elevates DA overflow.

Striatal KORs are also found on terminals of asymmetric, presumably excitatory synapses [149, 153]. As such, KORs are positioned to affect excitatory transmission. Consistent with localization of NAcc KORs on asymmetric synapses, in vitro electrophysiological studies have shown that KOR activation inhibits presynaptic glutamate release onto MSNs in NAcc [160, 161]. This finding is in accord with in vivo and in vitro neurochemical studies [162–165] demonstrating that KOR activation decreases evoked, but not basal, glutamate overflow. More specifically, KOR activation decreases Ca^{2+}-dependent, but not Ca^{2+}-independent, evoked glutamate overflow. However, inhibition of glutamate release onto MSNs by the selective KOR agonist, U69,593, was not blocked by Cd^{2+} or the non-selective, N-Type, and P/Q-Type Ca^{2+} channel blockers, ω-agatoxin, or ω-conotoxin, respectively, suggesting that KOR-mediated inhibition of release is not dependent on Ca^{2+} channel modulation [160]. These discrepancies may be explained by the use of different rat strains (Sprague–Dawley vs. Wistar) and/or animal age (adult vs. young) between studies. The presence of KOR-immunoreactivity in astrocytic processes [149] suggests that KOR activation may modulate glutamate reuptake by glia. This is of importance since dysfunction of glutamate reuptake and glutamatergic signaling has been implicated in addiction and other psychiatric disorders [166, 167].

Individual NAcc MSNs have extensive dendritic arborization and receive synaptic inputs from thousands of neurons from limbic, cortical, and thalamic sources, rendering MSNs integrative units that compute dendritic currents funneling into the soma [145]. KOR immunoreactivity is observed in somata and proximal dendrites of MSNs [149]. Immunoreactivity in somata is typically associated with cytosolic organelle membranes such as the endoplasmic reticulum and Golgi apparatus, while dendritic KORs are associated with the plasma membrane.

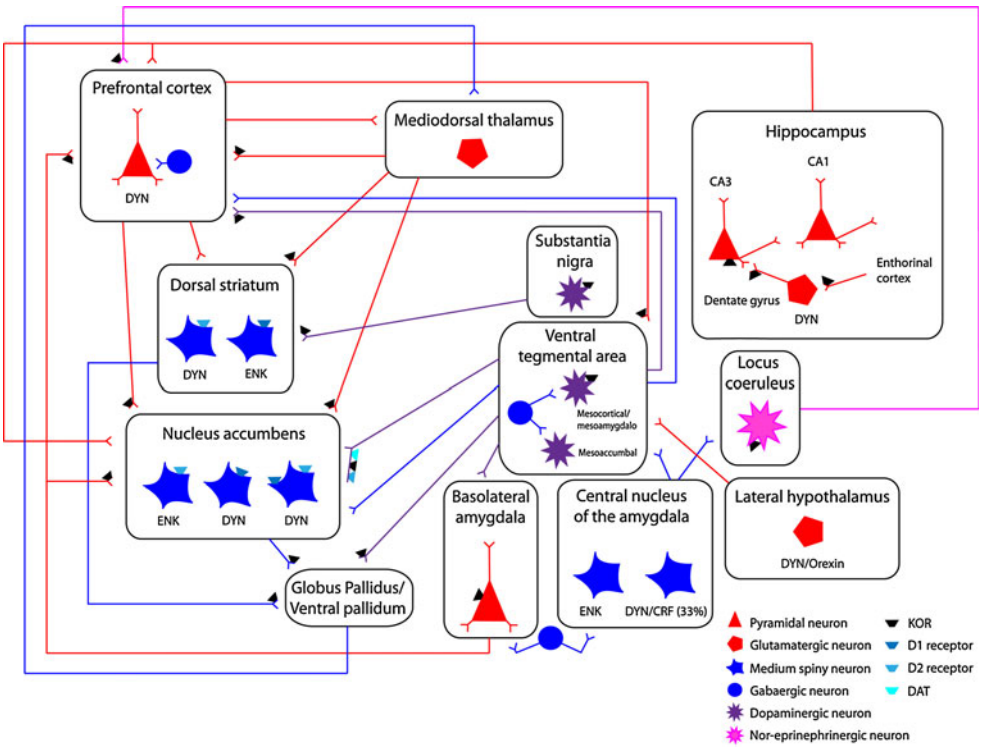

Fig. 2 The figure depicts a simplified scheme of neuronal circuits implicated in psychiatric disorders that are modulated by DYN/KOR pathways. DYN-positive neurons are located in the hypothalamus, central nucleus of the amygdala, cortical, and striatal regions and innervate neural substrates rich in KORs. DYN-positive neurons are innervated by glutamatergic and monoaminergic fibers containing presynaptic KORs, providing a physiological substrate for DYN/KOR to modulate presynaptic monoaminergic neurotransmission. The inset provides a key of neuronal/fiber type and simplified scheme of localization of KORs and DYN-positive neurons

KORs situated on proximal dendrites may be strategically situated to shape MSN computation of diverse dendritic signals via their slow, inhibitory action on neuronal excitability and Ca^{2+} signaling.

As previously mentioned, medial aspects of dorsal and ventral striatum receive dense glutamatergic afferents from cortical, thalamic, and limbic sources (see Fig. 2). It is not clear, however, whether presynaptic KORs differentially modulate each of these glutamatergic NAcc and dorsal-medial striatal inputs. This is important for our understanding of NAcc and striatal function since sustained activity may result in pathway-specific inhibition by DYN/KOR systems. Additionally, since glutamate is co-released with DA from VTA inputs [168–170], activation of KORs on DA terminals may inhibit glutamate release from vesicular pools in DAergic terminals. Moreover, presynaptic NAcc DA release may be modulated by glutamatergic NAcc afferents such as the basolateral amygdala (BLA) in a manner that is independent of VTA neuronal activity [171]. Thus, NAcc DYN activation of KORs in presynaptic excitatory terminals may further inhibit NAcc DA efflux via inhibition of local glutamate release that regulates impulse-independent DA efflux.

The activity of MSNs in the NAcc and dorsal striatum is governed by glutamatergic afferents arising in thalamic nuclei, limbic sites, and cortical regions, where DA modulates these excitatory afferents [172, 173]. DA acting on DYN-positive MSN neurons produces no functional consequence in the absence of glutamatergic synaptic transmission; rather DA acting on D1 and D2 DA receptors enhances and diminishes fast excitatory transmission mediated by glutamatergic receptors (i.e., NMDA receptors), respectively [174]. Ultrastructural studies have shown that, DYN-immunoreactive axon terminals appose KOR-immunoreactive terminals, DYN-containing KOR-immunoreactive terminals, and to a lesser extent soma/dendrites [149]. Additionally, PDYN and DYN are in close proximity to D1 receptors in dendrites and axons [175]. This suggests that DYN peptides are released from terminals and possibly from dendritic sites. However, it is unclear whether dendritic DYN release occurs in striatal nuclei, as is the case in other regions [176, 177]. The release of neuropeptide transmitters from dense-core vesicles (DCVs) requires sustained neuronal activity resulting in Ca^{2+} accumulation away from active zones of release where DCVs are located [178]. Therefore, the possibility exists that dendritic DYN release may be triggered by back-propagating action potentials, Ca^{2+} influx through NMDA receptors, and/or Ca^{2+} spikes. PDYN synthesis and peptide release in striatal compartments is positively regulated by DA D1 and NMDA receptors [179–182]. Intra-striatal perfusion of the partial D1 receptor agonist, SKF38393, produces a concentration-dependent increase in dynorphin B dialysate levels, which correlated with a decrease in DA levels [182], presumably due to dynorphin-mediated activation of KOR that regulate DA overflow. Additionally, MK-801, a non-competitive NMDA channel blocker, attenuates elevations in PDYN synthesis induced by DA receptor agonists [182]. These findings suggest that the dynorphin/KOR system provides feedback inhibition in response to DA and glutamate transmission in the ventral and dorsal striatum [183, 184]. Thus, under normal physiological conditions dynorphins may be released from MSNs when they receive concomitant DAergic and strong glutamatergic inputs.

Modulation of NAcc DA by KOR agonists and antagonists has traditionally been ascribed to KOR-mediated inhibition and disinhibition of DA release, respectively. However, KOR-mediated alterations in dorsal and ventral striatal DA overflow may also result from modulation of DAT and DA D2 autoreceptors. KORs positively regulate DAT function in striatum and NAcc with acute KOR activation increasing DAT function [154, 185], an effect that cannot be attributed to a circuit effect since analogous results are obtained in ex vivo studies [186]. Furthermore, our recent studies in cell expression systems have not only shown that KOR regulates DAT function but that these proteins are associated [186]. An anatomical basis for the interaction of these proteins is suggested by ultrastructural studies showing that KORs are apposed to DAT in NAcc nerve terminals [150]. Moreover, KORs may also play a role in modulating presynaptic D2-like DA autoreceptors, which regulate DA release and reuptake, since repeated KOR agonist administration downregulates presynaptic D2 DA receptor function [187]. Collectively, these studies suggest the DYN/KOR system is an important modulator of presynaptic striatal DA dynamics. Modulation results from (1) direct inhibitory actions of KORs on presynaptic DA release, (2) up-regulation of transporter function, and (3) possible regulation of presynaptic D2 DA receptors that mediate DA tone. This provides evidence that KOR in dorsal and ventral striatum may be a component of a larger macromolecular complex composed of KOR, DAT, and DA D2/D3 receptors, which tightly regulate DA dynamics in the striatum. Such receptor heteromers have been proposed to regulate neurotransmission [188, 189].

VTA/substantia nigra

Midbrain monoaminergic nuclei are innervated by afferents that express PDYN and/or KORs arising from limbic and "motor" neuronal regions [190]. The VTA and substantia nigra pars compacta (SNc) are composed of DAergic and GABAergic neurons that project to striatal, pallidal, prefrontal cortical, and limbic (amygdala) structures (see Fig. 2) [145]. Dorsal and ventral striatum GABAergic MSNs which express DYN [151, 152]

preferentially innervate midbrain structures including the VTA and SN [190]. This constitutes a part of the "direct pathway" of the basal ganglia. DYN pathways differentially innervate the VTA and SN depending on their origin [190]. The central nucleus of the amygdala (CeA), which contains DYN-positive striatal-like GABA neurons, sends moderate projections to the dorsal VTA, SN pars compacta, and brainstem. Additional lighter DYN inputs to the VTA and medial SN arise from the lateral and medial hypothalamus. Lateral hypothalamic neurons that express DYN also co-express orexin/hypocretin neuropeptides [191], which modulate VTA DA neuron activity [192]. Mesocortical and mesoamygdalo, but not mesolimbic, DA neurons are directly hyperpolarized by U69,593 [193, 194]. In agreement with these findings, in vivo microdialysis studies demonstrated that intra-VTA administration of KOR agonists does not decrease basal NAcc DA levels [155, 194, 195], but decreases medial PFC (mPFC) DA efflux [194]. Collectively, these studies provide evidence that whereas functional KOR expression is not present in mesoaccumbal DA soma, it is present in mesocortical DA cell bodies. At odds with these observations, U69,593 directly hyperpolarized mesolimbic and mesoamygdalo DA neurons and decreased DA-mediated inhibitory post-synaptic currents by inhibiting somatodendritic DA release in midbrain neurons from mice [196]. Factors that may account for the discrepancies include, but are not limited to, differences in species used (rat vs. mouse) and the particular neurons sampled in both studies. Administration of nor-BNI into the SNc via reverse dialysis increases local and dorsal striatal DA efflux, suggesting that KORs in the SNc tonically inhibit SNc DA neuron activity in vivo [159]. Thus, SNc KORs may differentially modulate nigrostriatal pathways as compared to mesocortical and mesolimbic DA pathways. In addition to direct action of KORs on DA neurons, KOR activation decreases VTA glutamate release [197]. Inhibition by presynaptic KORs is more robust onto cells directly hyperpolarized by the mu-opioid receptor (MOR) agonist, DAMGO, (putative GABAergic neuron), suggesting that endogenous DYN differentially regulates presynaptic glutamate release onto midbrain DA and GABAergic neurons. This may provide a physiological framework for opposing control of VTA DA neuron activity by MOR (DA neuron excitation) and KOR (DA neuron inhibition) systems that accounts for contrasting behavioral effects of these systems, although both inhibit VTA glutamate release.

Locus coeruleus

The locus coeruleus (LC) is the primary source of norepinephrine (NE) in the CNS and plays a major role in arousal, stress, and cognition [198, 199]. Dense DYN immunoreactivity is restricted to fibers interspersed within the LC (see Fig. 2) [144, 200]. Moreover, the majority of positive fibers are asymmetrical suggesting release at excitatory synapses [200]. KORs are localized on NE neuron somata and dendrites, presynaptic terminals co-expressing PDYN, and axons innervated by DYN-positive presynaptic terminals [201]. Thus, endogenous DYN may act on hetero-autoreceptors to inhibit presynaptic glutamate and/or aspartate release. Consistent with anatomical studies, in vitro electrophysiological studies have demonstrated that U50,488 and another synthetic KOR agonist, CI-977, decrease electrically stimulated excitatory post-synaptic potentials (EPSPs) in LC neurons, in a naloxone and nor-BNI-dependent manner [202, 203]. Moreover, neither agonist was effective in decreasing EPSPs elicited by pressure application of glutamic acid suggesting a pre-synaptic site of KOR action. Ionotophoretic application of the KOR agonist, U50,488, decreased excitation of LC neurons produced by stimuli known to excite LC neurons (sciatic nerve stimulation and opioid withdrawal) without changing tonic activity in vivo [204]. Collectively, these findings suggest that KORs may inhibit excitatory pre-synaptic LC inputs. Thus, DYN peptides acting on KORs may decrease synaptic transmission into the LC in response to sustained LC neuronal discharge during attentional, stressful, or arousing stimuli.

Amygdaloid structures

DYN is expressed in GABAergic neurons localized to lateral portions of the CeA. DYN-positive neurons are segregated from enkephalin-positive GABAergic neurons, which are localized to medial portions of the CeA (see Fig. 2) [144, 151]. These fibers innervate the VTA and LC. Electrolytic CeA lesions decrease DYN immunoreactivity in the LC suggesting that DYN-positive CeA neurons innervate the LC [205]. A moderate proportion of CeA DYN-containing neurons (approx. 30–40%) co-express corticotropin-releasing factor (CRF) but do not co-express enkephalin [205, 206]. Findings from ultrastructural experiments mirror the latter observations demonstrating that DYN immunoreactivity is co-localized in LC CRF-positive axon terminals [205]. This raises the possibility that CeA neurons co-release CRF and DYN in monoaminergic nuclei such as the LC and VTA. CRF can facilitate or inhibit excitatory synaptic transmission in the VTA [192]. Thus, sub-populations of DYN- and CRF-positive CeA neurons targeting VTA or LC (i.e., terminal regions rich in KORs and CRF receptors), may play a critical role in shaping synaptic activity in the VTA through concerted release of DYN and CRF. This modulation may "fine tune" DAergic neuron activity in an activity-dependent manner under normal physiological conditions.

Cortex

In situ hybridization and immunocytochemical studies have demonstrated that both cortical pyramidal and non-pyramidal cells (based on cell morphology) express PDYN and DYN peptides (see Fig. 2) [144, 207, 208]. Generally, expression is densest in layers II/III and V [144]. Dense KOR-positive neurons/fibers are observed in the same layers. Less dense staining is seen in all other layers but layer I [146, 209–211]. Given the role of layers II/III and V in intra- and extra-cortical neurotransmission, respectively, these localization studies suggest that the DYN/KOR system modulates diverse aspects of cortical information processing. With regards to cortical regions, species differences in density of DYN and KORs exist while the laminar distributions appear conserved. Although species differences are observed in relative expression of ligand and receptor between cortical regions, in situ hybridization and immunohistochemical studies have consistently shown high levels of DYN and KOR expression in the temporal and prefrontal cortex of rodents and primates relative to other cortices [147, 148] but substantial expression is also observed in other cortical regions.

KORs in the rat medial prefrontal cortex (mPFC) are localized on axonal varicosities [212]. The mPFC receives dense DA, NE, and serotonin (5-HT) varicose innervation from the VTA, LC, and dorsal raphe, respectively. KORs are also localized on presynaptic components of asymmetric synapses [212]. In vitro studies have demonstrated that KOR activation decreases [^{3}H] DA, [^{3}H] 5-HT, GABA, and glutamate release from mouse and rat synaptosomal preparations [213–215]. U50,488 decreases [^{3}H] NE release from human, but not rat neo-cortical slices [216]. Using in vivo microdialysis, we have recently demonstrated that mPFC KOR activation decreases local DA overflow [217]. Additionally, mPFC KORs tonically inhibit mPFC DA overflow. The effects of intra-mPFC KOR agonist administration on local 5-HT and NE release have not been investigated in vivo. It is currently not clear if KORs similarly modulate DAT or D2 autoreceptor function in the mPFC as in the dorsal and ventral striatum since expression of both DAT and D2 autoreceptors is substantially less in cortical regions. Additionally, reuptake of DA in mPFC is not solely handled by DAT, but rather by both DAT and the NE transporter [218], suggesting that KOR modulation of DA dynamics in this region may differ from striatal regions.

Hippocampus

For a detailed overview of the functional anatomical role of the DYN/KOR system in regulating hippocampal transmission, the reader is referred to a comprehensive review by Drake et al. [176]. DYN immunoreactivity in the hippocampus is densest in the mossy fiber pathway from the dentate gyrus to CA3 with little or no expression in other hippocampal regions [219, 220]. KOR activation by endogenous or exogenous ligands decreases mossy fiber pathway synaptic transmission in guinea pigs, Long–Evans rats, DBA/2 and C57BL/6 mice, and hamster [221–224], an effect that is absent in Sprague–Dawley rats [222]. This suggests the existence of strain differences in rats. Nor-BNI bath application promotes induction of LTP of the mossy fiber pathway when sub-threshold tetanic stimulation protocols are utilized [223]. DYN inhibition of mossy fiber transmission is enhanced after tetanic stimulation of mossy fibers and this enhancement of inhibition is specific to tetanized, but not non-tetanized, mossy fiber pathway synapses in the same preparation [223]. Moreover, DYN peptides are recruited to produce heterosynaptic inhibition in the mossy fiber pathway [221, 223]. Thus, DYNs released in response to strong stimuli may decrease function of mossy fiber pathway synapses that have low levels of activity, suggesting DYN/KOR modulation of synaptic transmission appears to be synapse-selective and highly dependent on activity. DYN released from dendrites and/or local collaterals of dentate gyrus granule cells may act in a retrograde fashion and activate KORs on perforant path terminals arising from the entorhinal cortex resulting in inhibition of entorhinal cortex inputs to the dentate gyrus [224, 226]. Retrograde signaling by DYN has been reported in the hypothalamus [39], suggesting that retrograde signaling may extend to other DYN-rich regions. Collectively, these studies provide evidence that DYNs are recruited by sustained mossy fiber or perforant path activity under normal physiological conditions to control information relayed to the hippocampus from the entorhinal cortex.

Recently, the radiolabeled tracers ^{11}C-GR103545 and MeJDTic, antagonists with high KOR affinity, were developed for positron emission topography, thus allowing in vivo assessment of KOR binding [227–229]. The results of studies using these ligands show a distribution of KORs in vivo that mirrors that of previous histological studies. These and other radiolabeled KOR ligands will enable investigation of KOR density/binding in healthy human volunteers and patients with psychiatric disorders. This will allow determination of whether there are alterations in steady-state KOR densities/binding. More importantly, these advances may eventually allow examination of KOR binding and DYN release (via a reduction in competitive radiotracer binding) during different behavioral states and in response to stimuli that exacerbate behavioral abnormalities in psychiatric disorders (i.e., stress).

Behavioral effects produced by KOR ligands in humans

In healthy humans, KOR agonists produce behavioral effects akin to those associated with schizophrenia, drug addiction, and bipolar disorder. For instance, intravenous administration of the preferential KOR agonist, (-) MR2034, produces psychotomimetic, anxiogenic, and sedative effects [229]. Psychotomimetic effects include perceptual distortion of sensory stimuli, depersonalization, speech and language impairments, and thought disorganization. Collectively, these behavioral effects may be perceived as dysphoric behavioral states. Importantly, these effects are stereo-selective and naloxone reversible, suggesting opioid receptor-mediation. Intramuscular administration of enadoline, a synthetic KOR agonist, produces effects perceived as "bad" in humans and psychotomimetic effects at higher doses [230]. Spriradoline, a synthetic KOR agonist, produces dysphoria and sedation [231–233], and psychotomimetic effects in some patients [233]. *Salvia divinorum* is a potent hallucinogenic herb increasingly used recreationally. The psychoactive compound in *S. divinorum* is salvinorin A, a selective, highly potent KOR agonist [234]. *S. divinorum* use in humans produces psychomimesis, sedation, speech and language impairments, and uncontrollable laughter, effects similar that of synthetic KOR ligands [235]. However, the effects are more rapid and transient as *S. divinorum* is usually smoked. Interestingly, a substantial proportion of *S. divinorum* users report that "entering another reality" (e.g., psychotomimesis) is the "best thing" about *S. divinorum* intoxication, while only a small proportion report unpleasant physical after-effects and "bad things" about intoxication [236]. This is in stark contrast to observations in laboratory settings where most subjects report dysphoric or unpleasant effects, suggesting that expectancy of a "trip" may determine whether the experience is deemed a positive or negative outcome. However, it should also be noted that most *S. divinorum* users have a high degree of cannabis and nicotine consumption [236]. Therefore, it is possible that self-reported effects of *S. divinorum* are influenced by interactions of several psychoactive compounds. Future research should be aimed at determining whether psychotomimetic effects produced by *S. divinorum* or other KOR agonists are synergistic when concomitantly consumed with cannabis. This is of particular importance since cannabinoids can have pro-psychotic and anti-psychotic effects, depending on the cannabis composition and developmental period (e.g., adolescence) of exposure [237, 238]. Recently, case reports have described recurrent psychosis-like symptoms days after *S. divinorum* toxicity in adolescents [239–241], which could precipitate symptoms of psychiatric disorders not under the direct pharmacological influence of *S. divinorum*. Recurrent psychosis is likely not frequent in *S. divinorum* users, but may be exhibited in subset of users with genetic predisposition or heightened risk to develop schizophrenia or other affective disorders.

Behavioral effects of KOR ligands relevant to psychiatric disorders

KOR ligands and conditioned-aversive effects

Pavlovian procedures, such as the conditioned place aversion (CPA) paradigm, have been utilized to determine the motivational effects of KOR ligands in rodents. In these procedures, drug is repeatedly paired with a conditioned stimuli (CS^+; i.e., a discrete compartment) and approach (i.e., preference) or avoidance (i.e., aversion) behavior to the CS^+ in the absence of drug is assessed during testing. Mucha and Herz [242] first demonstrated that systemic KOR agonist administration produces conditioned aversive effects in rodents using CPA and conditioned taste aversion paradigms. These conditioned aversive effects have been suggested to be mediated by KOR agonist-induced "dysphoria". However, dysphoria cannot be measured in rodents. In light of the wide range of behavioral alterations KOR agonists produce, the possibility exists that psychotomimetic-like effects, anxiety-like behavior, and/or sedation may contribute to the conditioned aversive effects of KOR agonists in rodents. Microinjection of U50,488 into medial PFC, midbrain, NAcc, or lateral hypothalamus, but not to the SN or dorsal striatum, is sufficient to produce CPA [243]. This suggests that KORs in the mesolimbic DA pathway (VTA and NAcc) and limbic sites may be responsible for conditioned aversive effects produced by systemically administered KOR agonists. At odds with these findings, a recent study demonstrated that systemic, low-dose salvinorin A administration (10–40 μg/kg) produces conditioned place preference [244]. This is in contrast to the aversive effects produced by higher doses (160–3,000 μg/kg) [156, 244, 245]. Interestingly, systemic administration of a salvinorin A dose that produces CPP enhances DA overflow in the NAcc shell [244]. In contrast, higher doses decrease NAcc DA efflux [245, 246]. The conditioned rewarding effects of salvinorin A were blocked by rimonabant, a CB1 receptor antagonist as well as nor-BNI, suggesting that endogenous cannabinoid signaling may contribute to the positive conditioned effects of low-dose salvinorin A. Evidence that salvinorin A produces anxiolytic effects and decreases immobility in the forced swim test at low doses has also been obtained [247].

KOR-ligands and intracranial self-stimulation

Intracranial self-stimulation (ICSS) procedures are sensitive to manipulations that decrease motivation or decrease brain reward function [248]. Rate-frequency ICSS procedures have been widely utilized to assess the motivational and anhedonic effects of drugs. In this paradigm, stable ICSS thresholds are established and persist for days to weeks. Anhedonic behavioral states (i.e., drug withdrawal) or drugs that produce conditioned aversive effects reliably increase ICSS thresholds, suggesting that more ICSS is needed to reach "normal" brain reward levels. Systemic administration of KOR agonists increases thresholds in rats [245, 249, 250], whereas KOR antagonists are without effect, suggesting that endogenous KOR signaling does not tonically inhibit brain reward function [250, 251]. Collectively, Pavlovian and ICSS procedures demonstrate that activation of DYN/KOR systems produce negative motivational effects. However, mimicking a psychiatric behavioral phenotype does not imply that DYN/KOR dysregulation produces negative affect and anhedonia in these disorders. Future research aimed at determining whether this system contributes to negative affect/mood or anhedonia in psychiatric disease is warranted.

KOR/DA interactions and conditioned aversion

The ability of systemic KOR activation to produce CPA is dependent on intact NAcc DA signaling since DA D1 receptor antagonism and mesolimbic, but not nigrostriatal, DA pathway denervation blocks KOR agonist-induced CPA [252, 253]. NAcc KOR activation decreases extracellular DA levels by decreasing Ca^{2+}-dependent release [163], and increasing DA uptake [153, 184]. These effects are consistent with findings that decreasing DA neuronal activity produces CPA [254, 255]. Thus, modulation of NAcc DA is a mechanism by which DYN/KORs exert negative motivational effects.

KOR-serotonin (5-HT) interactions and conditioned aversion

The conditioned aversive effects of KOR ligands may also be mediated by interactions with 5-HT systems. U50,488-induced CPA was blocked by intra-dorsal raphe nuclei (DRN) microinjections of nor-BNI, suggesting that DRN KOR activation is necessary for U50,488-mediated aversion [256]. In this study, selective expression of KORs in the DRN of *oprk1* knock-out mice enabled systemic U50,488 to produce a CPA. This effect is blocked by NAcc microinjection of nor-BNI, suggesting that KOR agonists produce negative affect by modulating 5-HT signaling from the DRN to the NAcc. However, the extent to which KOR overexpression is physiologically relevant is unclear. Neurochemical studies have demonstrated that administration of U50,488 into the NAcc or DRN via-reverse microdialysis decreases terminal and somatodendritic5-HT overflow, respectively [257, 258]. As previously mentioned, KOR and D2 autoreceptors regulate monoamine function by affecting monoamine transporter function. Therefore, it is imperative to determine whether effects of KOR activation on 5-HT overflow are mediated by KOR regulation of 5-HT release and/or 5-HT transporter function. Indeed, evidence that KOR activation affects 5-HT transporter function in cells and native tissue has recently been presented [259]. Inconsistent with the notion that 5-HT neurotransmission mediates the conditioned aversive effects of KOR agonists, experimental manipulations that decrease DRN 5-HT neuron function produce reward [260]. For example, intra-DRN microinjection of GABA receptor agonists, glutamate antagonists or 5-HT1A receptor (a presynaptic 5-HT autoreceptor) agonists produce place preference and facilitate ICSS, suggesting that DRN 5-HTergic function tonically inhibits reward. Additionally, rats will readily self-administer GABA agonists and glutamate receptor antagonists into the DRN [260]. However, these manipulations decrease 5-HT neurotransmission not only in the NAcc, but, in a plethora of terminal regions (i.e., medial PFC) such that these effects may be mediated via reduced 5-HT efflux in regions other than the NAcc. Interestingly, doses of systemic salvinorin A that increase ICSS thresholds do not decrease NAcc 5-HT extracellular levels [245], suggesting that changes in 5-HT transmission may not be associated with anhedonia/negative affect produced by systemic KOR agonist administration. Future work should be aimed at determining whether the interactions between the KOR and 5-HT system are direct or indirect via 5-HT interactions with other neurotransmitter systems.

DYN/KORs, stress, and "pro-depressant-like" effects in animal models

Although acute stress is beneficial for survival by recruiting appropriate motivational and cognitive processes, chronic stress can produce long-lasting alterations in affect and mood, anhedonia, and cognitive deficits; behaviors that are relevant to depression and other psychiatric disorders. Learned helplessness and repeated forced swim stress robustly increases DYN immunoreactivity in hippocampus (CA3, dentate gyrus) and NAcc [261]. Increased hypothalamic and decreased striatal DYN A immunoreactivity after context-induced immobility (paired with electric shock) and a single forced swim exposure have also been reported [262]. Thus, KOR systems may initially recruit stress systems by stimulating the hypothalamic–pituitary

adrenal (HPA) axis. CRF is a powerful mediator of behavioral stress responses and its dysregulation is implicated in depression, bipolar disorder, addiction, and schizophrenia [263]. CRF promotes DA-dependent release of DYN peptides in striatal regions [264, 265]. Moreover, enhanced phospho-KOR immunoreactivity, an index of KOR activation, is increased in the NAcc, hippocampus, BLA, bed nucleus of the stria terminalis, DRN, VTA, and ventral pallidum following central CRF administration [266]. Together, these data indicate that repeated stress and the ensuing increase in CRF enhance DYN release and KOR activation which then modulates stress reactivity. Consistent with this view, systemically administered KOR agonists increase immobility in repeated forced swim procedures whereas rodents treated with KOR antagonists and *pdyn* KO mice exhibit decreased immobility [251, 261, 267, 268].

KOR antagonists produce effects similar to that of traditional anti-depressants [251, 268, 269]. In the forced swim test, rodents are subjected to repeated forced swim over a 2-day period and measures of immobility (i.e., latency to immobility or time spent immobile) increase with repeated swim trials, an effect interpreted as increased "behavioral despair" that is indicative of "depressive-like" behavior [270]. Therefore, effects produced by KOR agonists and antagonists have been interpreted as "pro-depressive" and "anti-depressant", respectively. Animal models of depression typically involve exposing subjects repeatedly to a stressful event (i.e., forced swim) from which there is no escape. Depression is characterized by persistent negative mood/affect, anhedonia, decreased motivation, and cognitive deficits. Anti-depressants widely used to treat depression target monoamine transporters. Importantly, however, therapeutic efficacy requires repeated anti-depressant treatment, suggesting that long-term alterations in neural circuitry function rather than direct pharmacological effects of antidepressants (elevating extracellular monoamine levels) mediate therapeutic efficacy. Recently, the relevance of several animal models of depression (forced swim and tail suspension tests) has been called into question due to limited face and predictive validity [271]. Indeed, in these models, antidepressant efficacy is observed after acute administration. Social defeat stress is an animal model widely used to model depression. Therapeutic efficacy with typical antidepressant is observed after chronic, but not acute, treatment [271]. Nor-BNI-treated wild-type mice exhibit decreased social defeat-induced behavior (i.e., defeat postures), suggesting that endogenous DYN release mediates stress-induced "pro-depressive-like" effects [272]. This is of interest, since in the social defeat stress model the "anti-depressant" phenotype of nor-BNI-treated mice is observed after short-term nor-BNI treatment, in contrast to the effects of typical anti-depressants. Questions, thus, remain as to whether the effects of KOR ligands observed in some animal models of depression (i.e., forced swim stress) have direct relevance to depression. Additionally, in controlled laboratory studies in humans, spiradoline did not produce depressive effects although sedation and dysphoria were reported [233]. Importantly, the aforementioned models of depression produce stress and as such may be relevant to furthering our understanding of the role of the DYN/KOR systems in stress, which is known to exacerbate behavioral abnormalities in depression and other psychiatric disorders.

Interestingly, the ability of forced swim stress and central CRF infusion to produce aversion to an odorant or compartment to which it is discretely paired is absent in wild-type mice pretreated with nor-BNI and in *pdyn* knockouts [266]. This suggests that DYN activation of KOR not only contributes to stress reactivity but to the conditioned aversive effects of stress and CRF receptor activation. Stressful stimuli and CRF increases monoaminergic and glutamatergic transmission in limbic and cortical structures such as the hippocampus, mPFC, and NAcc [191]. The possibility exists that repeated stress increases monoamine and excitatory neurotransmission in these regions resulting in activity-dependent DYN release and KOR activation, and, ultimately long-lasting changes in neuronal activity in structures that regulate affect.

Neonatal stress recapitulates many facets of psychiatric disorders including anhedonia and alterations in affect and mood that persist through adulthood [273]. Neonatal maternal separation, a stressor, profoundly affects DYN peptides and phosphor-KOR immunoreactivity in regions that mediate stress responses such as the hippocampus, amygdala, hypothalamus, mPFC, and pituitary that persist into adulthood [274, 275]. Consistent with the hypothesis that neonatal stress enhances KOR signaling during adulthood, neonatal maternal separation enhances CPA produced by U50,488 during adulthood [276]. Thus, neonatal stress in rodents produces enduring alterations in DYN/KOR systems that may contribute to abnormal behavioral responses that are characteristic of a variety of psychiatric disorders and which are exacerbated by stress.

DYN/KOR alterations in human post-mortem tissue

The striatum has a "patch" and "matrix" organization, with patches richer in DYN-positive neurons than the surrounding matrix [277]. *PDYN* mRNA is elevated in patch compartments in post-mortem tissue of suicide subjects, an effect presumably attributed to depression [278]. As previously mentioned, similar alterations are present in animal models with a repeated stress components [279]. However, the underlying psychiatric disorder/s of the

human subjects is not clear. No significant differences in either *PDYN* or *OPRK1* mRNA levels in cingulate and dorsal lateral prefrontal cortex of patients with bipolar disorder or major depression were seen relative to controls [280]. However, *PDYN* expression is reduced in the amygdala of individuals suffering from depression or bipolar disorder [281]. Although these data suggest that PDYN synthesis and/or turnover is altered, the functional consequence of this decrease is not known.

CREB and the DYN/KOR system

CREB regulates *pdyn* expression in rodents (see trans-regulation section). Region specific alterations in CREB function is observed following stress exposure, antidepressant treatment, and in animal models of depression [282]. CREB and dominant negative CREB (mCREB) overexpression in the NAcc increases and decreases immobility in the forced swim test, respectively [269, 283]. Interestingly, the effects of CREB overexpression are ameliorated by central KOR blockade [269], suggesting that enhanced DYN mediates the effects of CREB overexpression. Importantly, nor-BNI decreased immobility in controls, NAcc CREB over-expressing, and NAcc mCREB over-expressing rats. Moreover, the effect of mCREB overexpression on forced swim is associated with decreased *pdyn* expression in mCREB-expressing MSNs, suggesting that decreased DYN signaling is associated with decreased immobility [283]. Intracerebroventricular and intra-accumbal nor-BNI, but not intra-dentate gyrus, treatment mimics the effects of mCREB overexpression on forced swim [283]. Moreover, elevations in ICSS thresholds produced by systemic U50,488 administration are absent in mCREB mice, suggesting that the "antidepressant" phenotype of these animals may be related to decreased KOR function [284]. However, the mechanism by which mCREB expression abolishes effects produced by KOR activation on ICSS behavior is not clear. Taken together, these studies demonstrate that enhanced NAcc DYN expression by CREB increases KOR signaling and alteration in behavior relevant to psychiatric disorders.

DYN/KOR systems and anxiety

Preclinical studies are consistent with clinical work demonstrating that KOR agonists increase anxiety [229]. Systemic KOR antagonist administration produces anxiolytic effects in elevated plus-maze, open-field, and fear-potentiated startle paradigms in rats [285], suggesting that endogenous DYN release mediates the expression of anxiety-like behavior in these behavioral paradigms. *Pdyn* knock-out mice exhibit decreased anxiety-like behavior in open-field, elevated plus-maze, and light/dark box paradigm. Similar effects are observed in wild-type mice pretreated with selective KOR antagonists [286]. However, inconsistent with these observations, *oprk1* knock-out mice do not display altered anxiety-like behavior in these tests [287]. Consistent with the notion that DYN release occurs in response to anxiogenic environmental stimuli, systemic nor-BNI administration or *pdyn* ablation decreases anxiety-like behavior in procedures involving anxiety-eliciting testing conditions (brightly lit testing conditions) [288], an effect not present under normal testing conditions. Moreover, U50,488 administration produces anxiogenic effects in the elevated plus-maze and BLA KOR blockade reverses the anxiogenic effects of stress and central CRF administration. This is consistent with work demonstrating that intra-amygdala microinjections of DYN A increase anxiety-like behavior in the light–dark box test [289], and suggests that BLA DYN signaling is also a downstream mediator of the anxiogenic effects of stress and CRF. Human studies examining whether KOR antagonists decrease basal and stimulated anxiety is warranted in view of the potential implications of these findings for panic/anxiety disorders.

Wistar–Kyoto rats display enhanced stress reactivity and anxiety-like behavior relative to Sprague–Dawley rats and are considered an animal model of depression or anxiety disorders [267]. Wistar–Kyoto rats are more sensitive to the anxiolytic and stress-ameliorating effects of KOR blockade [267, 290], suggesting there is enhanced DYN/KOR signaling in this strain. Indeed, enhanced KOR- and DYN A-immunoreactivity is observed in the piriform cortex and NAcc, respectively, of Wistar–Kyoto relative to Sprague–Dawley rats [267]. Moreover, intra-piriform cortex nor-BNI administration ameliorates depressive-like behavior in Wistar–Kyoto rats. Collectively, these studies suggest that genetic differences in the DYN/KOR system may contribute to the predisposition to depressive- and anxiety-like phenotypes. As such, KOR antagonists may be useful antidepressants in discrete sub-populations with major depression and/or anxiety/panic disorders.

DYN/KOR and addiction

Drug addiction is a chronically relapsing disorder characterized by preoccupation with drug seeking and intake despite the aversive consequences that may ensue. Chronic drug use is also associated with the development of tolerance and a characteristic withdrawal syndrome. Initially, the rewarding properties of drugs of abuse drive behavior. With continued use, drug seeking/taking is driven by a balance between the positive effects produced by the drug, avoidance of the negative consequences of drug withdrawal and increased salience of drug-associated stimuli

[291]. In addition, stress and conditioned stimuli associated with drug availability are capable of reinstating compulsive drug seeking and taking. The DYN/KOR system has been implicated in the development of drug addiction [183, 279, 292, 293]. Additionally, there is co-morbidity of substance use disorders and other psychiatric disorders [294]. Thus, it has been suggested that DYN/KOR dysregulation contributes to aberrant activity in brain regions that influence drug addiction and behavioral alterations in psychiatric disorders (i.e., motivational processes).

DYN/KOR system alterations in human post-mortem tissue

Since polysubstance use is common among drug addicts and drug use pattern is often unknown, it is difficult to parse the effects of individual drugs in humans. *PDYN* expression is enhanced in putamen "patches" but not in caudate or NAcc of cocaine users, whereas [^{3}H]DYN binding is increased in the caudate relative to controls [277]. Enhanced DYN immunoreactivity in the caudate and ventral pallidum but no changes in the putamen and prefrontal cortex of methamphetamine and cocaine users have been reported [295, 296]. Methamphetamine users have decreased DYN immunoreactivity in the NAcc, medial pulvinar thalamic nucleus, and temporal/occipital association cortices [296]; changes not observed in cocaine users [295]. A significant correlation between recent psychostimulant use and *PDYN* expression in cingulate and dorsal lateral prefrontal cortices has also been reported [280]. Additionally, past, but not recent, marijuana use was associated with increased expression in these regions, suggesting that the time course of *PDYN* induction may vary depending on drug of abuse. Although studies to date suggest that DYN/KOR systems are altered with drug use, given limitations of post-mortem studies in humans, the only solid conclusion that can be made is that striatal *PDYN* expression is increased in psychostimulant users.

Cocaine and amphetamines/preclinical studies

The role of DYN/KOR systems in psychostimulant-induced drug seeking and neurochemical alterations has been extensively studied. Cocaine blocks DA, NE, and 5-HT transporters producing elevations in extracellular monoamine levels in monoaminergic nuclei (i.e., VTA) and their terminal regions (i.e., mPFC). Amphetamine, like cocaine, is a monoamine transporter substrate, but also produces reverse transport of monoamines. Both drugs produce robust elevations in extracellular monoamine levels. The ability of psychostimulants and other drugs to increase DA and monoamine levels in the NAcc and other reward-related regions (i.e., mPFC) is implicated in mediating the rewarding properties of abused drugs [297, 298].

Psychostimulant-induced changes in the DYN/KOR systems in experimental animal models

Both acute and repeated psychostimulant administration increase *pdyn* expression in reward-related neuronal regions in animal models, similar to what is observed in humans [151, 181]. In rodents, administration of a single or repeated injection of cocaine or amphetamine produces robust elevations in *pdyn* mRNA [299–301] and DYN immunoreactivity [302, 303] in the NAcc and dorsal striatum. However, cocaine-induced NAcc *pdyn* induction is not as robust as that resulting from amphetamine [299]. In non-human primates, high-dose cocaine self-administration acquisition and chronic high-dose cocaine, but not low-dose, self-administration increases *pdyn* expression in the rostral caudate and putamen [304]. In rodents, cocaine self-administration increases dorsal lateral and dorsal medial striatal, but not NAcc or limbic, *pdyn* expression to a similar extent in yoked and self-administering rats [305]. This suggests that *pdyn* elevations are due to pharmacological effects of cocaine rather than drug taking behavior per se. The effects of psychostimulants on striatal *pdyn* expression are dependent on D1 DA receptor signaling such that D1 receptor antagonism [306] or D1 receptor deletion [178, 302] abolish psychostimulant-induced increases in *pdyn* expression. Importantly, psychostimulants also elevate extracellular DYN levels in the striatum and SN [307] suggesting increased pdyn synthesis. As previously mentioned, striatal D1- and NMDA receptor interactions may play an important role in information processing [172, 173] and the regulation of DYN synthesis [181]. Thus, it is not surprising that NMDA receptor antagonism blocks the ability of psychostimulants to elevate DYN immunoreactivity in dorsal and ventral striatal compartments [181]. The PDYN increase is postulated to be a compensatory mechanism to reduce psychostimulant-induced MSN activity by activating presynaptic KORs on excitatory synapses, DAergic varicosities, and in subsets of MSNs expressing dendritic KORs [151, 308]. This hypothesis is supported by findings that nor-BNI treatment or constitutive KOR deletion enhance cocaine-evoked NAcc DA dialysate levels [153]. Importantly, nor-BNI-treated wild-type mice as well as *pdyn* and *oprk1* knock-out mice display enhanced locomotor sensitization in response to cocaine treatment relative to controls [153, 309], suggesting that increased activity of DYN/KOR systems is a negative feedback mechanism opposing neurochemical changes produced by cocaine (i.e., elevations in DA and glutamate in the NAcc).

The influence of psychostimulants on KOR immunoreactivity and binding remains controversial. One study

reported decreased KOR density in dorsal striatum after acute or repeated cocaine injections whereas decreased NAcc KOR density was only observed after repeated cocaine exposure [299]. These changes may reflect a compensatory downregulation of KORs in response to *pdyn* induction. However, acute and repeated amphetamine treatment decreases KOR density in NAcc, but not striatum [299], where increased *pdyn* induction is typically observed. Examination of [^{3}H] bremazocine binding (in the presence of cold agonists to block other opioid receptors) 30 min after the last cocaine injection of a binge-like dosing regimen revealed increased KOR density in cingulate cortex, dorsal striatum, olfactory tubercles, and VTA. Using [^{3}H] CI-977 and an escalating cocaine treatment regimen, however, the same group only found a significant increase in septal KOR [310]. Acute high-dose, "binge" cocaine administration decreased *oprk1* mRNA in the SN [301]. However, after escalating doses of cocaine, KOR-coupling to $G_{i/o}$ G-proteins increased in the VTA, as assessed by [^{35}S] GTP binding [311]. Together, these studies suggest that changes in DYN/KOR systems are dynamic and vary according to the stage of the addiction cycle. Furthermore, it is apparent that this opioid system is recruited during normal physiological processes, but recruitment is exacerbated by psychostimulants.

Psychostimulant exposure alters the behavioral and electrophysiological effects of KOR agonists. U69,593-induced CPA is exacerbated for at least 10 days in rodents pretreated with a single cocaine injection and this effect is blocked by VTA inactivation [312], suggesting that DYN/KOR systems regulating mesocortical and/or mesolimbic neurotransmission are dysregulated following acute cocaine exposure. Indeed, the ability of DYN A and U69,593 to inhibit glutamatergic transmission in the NAcc, but not in the VTA, is disrupted during abstinence from acute amphetamine [313] or repeated cocaine injection [314]. Additionally, amphetamine-induced downregulation of KOR-mediated inhibition of glutamate transmission is reversed by concomitant amphetamine treatment with a DA D1 receptor antagonist or naltrexone [313]. These findings suggest that cocaine- and amphetamine-induced attenuation of KOR-mediated inhibition is due to D1 receptor-mediated release of endogenous DYN that downregulates NAcc KOR function. These studies also provide evidence that psychostimulants can produce functional changes in NAcc KOR systems in the absence of changes in *pdyn* expression or KOR density.

Anti-psychostimulant effects of KOR agonists

Endogenous DYN/KOR systems can act as inhibitory feedback systems recruited by psychostimulants. When the "temporal order" is switched and KORs are stimulated with agonists prior to psychostimulant administration, the psychostimulant-induced behavioral, neurochemical, and molecular effects are diminished. Pretreatment with synthetic or naturally occurring KOR agonists (15–20 min prior) decreases the behavioral- and locomotor-activating effects of acute and sensitizing-regimens of systemic cocaine [156, 315–318] and amphetamine [163, 300, 319]. Acute KOR agonist administration 15–20 min prior to conditioning decreases cocaine-induced CPP in rats [320] and mice [318, 321, 322], suggesting that KOR agonists decrease the conditioned rewarding effects of psychostimulants. Prior, repeated, home cage injections of KOR agonists attenuate the subsequent development of sensitization to the conditioned reinforcing effects of cocaine [323]. This effect cannot be attributed to the aversive effects of KOR agonists or a generalized disruption of learning or memory processes since sensitization to morphine is unaltered. A recent study has shown that U69,593 doses that are ineffective in altering ICSS thresholds in drug naïve animals, block cocaine-evoked decreases in ICSS thresholds [324]. It appears likely that these actions result from the ability of KOR agonists to decrease psychostimulant-evoked increases in NAcc and dorsal striatal DA and glutamate dialysate levels [157, 163, 318, 321]. Interestingly, Thompson and colleagues [182] demonstrated that repeated cocaine administration increases NAcc DA uptake, an effect that is blocked by U69,593 treatment. Repeated co-administration of U69,593 with cocaine blocks the increased DA uptake and decreased K^+-stimulated DA release in the mPFC associated with early abstinence from repeated cocaine [325]. Given these findings, the question arises as to whether KOR agonist treatment may attenuate alterations in mPFC-dependent cognitive function produced by cocaine.

Induction of the immediate early gene, *fos*, in neurons is an indirect marker of persistent neuronal activity and plasticity. Acute psychostimulant administration increases *fos* and other immediate early genes in the NAcc and prefrontal areas, and this effect is decreased by KOR agonists [151, 300]. Such findings may be of potential clinical relevance since C-FOS-positive NAcc neurons are implicated in the development of context-dependent cocaine sensitization [326]. Interestingly, *oprk1* knock-out mice display decreased cocaine-induced induction of C-FOS and FOS B [153]. U69,593 pretreatment decreased cocaine-induced elevations in DA and cyclic AMP-regulated phosphoprotein (DARPP-32), a protein involved in D1 receptor signal transduction, in hippocampus, dorsal striatum, and mPFC [327]. These studies suggest that KOR agonists antagonize the actions of psychostimulants when administered shortly before psychostimulant use presumably by countering neurochemical (i.e., DA-elevating effects of psychostimulants) and molecular

alterations that produce long-lasting plastic plasticity in reward-related structures. Evidence that the interaction of KOR agonists with psychostimulants depends on 5-HT signaling has also been obtained. Pretreatment with DL-*p*-chloroamphetamine, which depletes 5-HT stores, blocked the ability of U69,593 to decrease cocaine-stimulated locomotor activity [317], suggesting that the ability of KOR agonism to block cocaine-induced alterations in locomotor activity are partially dependent on 5-HT systems.

KORs and psychostimulant self-administration

Reports on the effects of KOR ligands on psychostimulant administration are conflicting. U50,488, spiradoline, and cyclazocine dose-dependently decrease low-dose cocaine self-administration (0.1 mg/kg) maintained under an FR1 schedule [328, 329]. This effect is stereo-selective [329] and nor-BNI reversible [328]. Importantly, responding for a natural reward (e.g., water) was only altered by high doses of U50,488 and spiradoline. Schenk et al. reported that U69,593 administration decreased self-administration of low cocaine doses (0.03–0.125 mg/kg) more robustly when cocaine doses were presented in a descending order, relative to conditions where doses were presented in a descending order [330]. Additionally, U69,593 was ineffective in attenuating self-administration of high doses of cocaine (>0.25 mg/kg). One explanation for the effect of KOR agonists on cocaine self-administration is that agonist-induced aversive or sedative effects disrupt self-administration. Indeed, chronic KOR agonists (spiradoline, bremazocine, but not cyclazocine) treatment (up to 69 injections of KOR agonists/day) decreased food-maintained responding in non-human primates [331]. However, systemic administration of KOR agonists at doses that did not alter responding for natural reinforcers was also shown to decrease cocaine self-administration [328, 331], and this may be ligand-specific [331]. Intra-VTA U50,488 microinjection does not alter responses for cocaine maintained under a FR5 schedule [332], suggesting that maintenance of cocaine self-administration is not modulated by VTA KORs. Systemic nor-BNI administration does not alter cocaine self-administration in rats under an FR1 schedule [328, 333]. However, Wee et al. using a progressive ratio schedule, recently demonstrated that a high dose of nor-BNI decreases cocaine self-administration break-points in rats with long access to cocaine, but not rats with short access. By contrast, this effect was not observed under fixed-ratio schedules [333]. This highlights the notion that the role of DYN/KORs in psychostimulant addiction is dynamic and may vary with the pattern of drug intake and duration of drug exposure.

KORs and CS^+ interactions in cocaine self-administration procedures

Systemic U69,593 administration to rats produces a long-lasting decrease in cocaine-seeking behavior in a paradigm in which a cue light is associated with cocaine infusion during training and testing. However, the effect of the agonist is transient in rats that acquired cocaine responding not associated with cue light activation [334]. This work suggests that KOR agonism does not simply block the unconditioned effects of cocaine or decrease overall motivation, but rather modulates the conditioned effects of cocaine. In agreement with the latter report, recent work from our laboratory using second order schedules of cocaine self-administration has demonstrated that systemic nor-BNI increases CS^+ maintained cocaine-seeking behavior but not cocaine-taking behavior maintained by both the CS^+ and pharmacological effects of cocaine [335]. This suggests that endogenous KOR signaling may play a key role in modulating CS^+-maintained responding. DA signaling in ventral–dorsal striatal loops is necessary for the expression of "habitual" drug seeking. Thus, one mechanism by which endogenous and exogenous KOR ligands may affect CS^+ maintained behavior is through modulation of dorsal striatal DA and glutamatergic efflux. BLA function is necessary for Pavlovian conditioning. Therefore, the effects of KOR ligands may be dependent on modulation of neurotransmission in the BLA or its afferents (e.g., mPFC or NAcc). Indeed, KOR agonism decreases stimulated field excitatory potentials and synaptic plasticity in the BLA [336] providing a potential mechanisms by which endogenous and exogenous KOR agonists may decrease the conditioned effects of cocaine.

KORs and psychostimulant-primed reinstatement

Systemic administration of KOR agonists (U69,593, enadoline, and spiradoline) decreases cocaine-primed reinstatement in rodents and non-human primates [330, 337, 338]. In contrast, amphetamine-primed reinstatement in rodents is unaffected [330]. There is no evidence that KOR antagonists alter cocaine-induced self-administration or the reinstatement of CPP produced by a priming injection of cocaine [337–339]. Interestingly, KOR agonists do not alter reinstatement produced by selective DAT inhibitors [338], suggesting that KOR agonist modulation of cocaine may be mediated by interactions with other monoamine systems, including 5-HT and NE. Additionally, U69,593 is more effective at attenuating cocaine-induced reinstatement than amphetamine-induced reinstatement [335]. As previously mentioned KORs are believed to

modulate monoamine transporter function. Thus subtle differences in drug action between cocaine (monoamine transporter blocker) versus amphetamine (monoamine transporter reverser) may explain why KOR agonists are more effective in attenuating cocaine-induced reinstatement. Intra-VTA microinjection of U50,488 decreases cocaine-primed cocaine reinstatement in a dose-dependent manner, and this effect was blocked by intra-VTA nor-BNI microinjection [332]. Intra-VTA U50,488 was without effect on cocaine and food responding after acquisition as well as on food-induced reinstatement of food self-administration. This suggests that effects of intra-VTA KOR administration on cocaine-primed reinstatement is specific and not due to changes in motivation or locomotor activity.

KORs and stress-induced cocaine reward and reinstatement

Stress associated with repeated forced swim or social defeat enhances cocaine-induced CPP and this effect is absent in nor-BNI-treated wild-type mice and *pdyn* knock-outs [268, 272]. These findings are consistent with the hypothesis that endogenous KOR systems are recruited by stressful events and enhance the motivational valence of cocaine [288]. U50,488 administered 15 min prior to conditioning sessions with cocaine or to CPP post-testing blocked whereas 60 min pretreatment potentiated cocaine CPP [322, 339], suggesting that the interaction of KOR agonists with cocaine is time-dependent. Data suggesting an involvement of DYN/KOR systems in mediating stress-induced reinstatement of cocaine-seeking behavior has also been obtained. Self-administration studies using a fixed ratio reinforcement schedule revealed an attenuation of stress-induced reinstatement of cocaine-seeking by KOR antagonists [340, 341]. Stress-induced reinstatement of cocaine CPP is also attenuated by KOR antagonism and in *pdyn* and *oprk1* knock-out mice [342, 343]. KOR agonists also reinstate cocaine self-administration in non-human primates [344]. Interestingly, clonidine, an α_2 nor-epinephrine autoreceptor and post-synaptic receptor agonist attenuated the effects of KOR agonists, suggesting that KORs may in part increase cocaine self-administration by increasing NE efflux in reward-related structures that are sensitive to stress. However, in the latter study the ability of KOR agonists to reinstate self-administration was blocked by naltrexone but not nor-BNI, suggesting a non-KOR opioid receptor-mediated effect. Analogous to reinstatement produced by a priming injection of cocaine, recent data suggest that KOR modulation of 5-HT may contribute to the stress/KOR agonists interaction. Land et al. [256] demonstrated that intra-DRN nor-BNI microinjection blocks social defeat stress-induced cocaine CPP reinstatement. This is of importance since 5-HTergic DRN neurons send efferent projections to brain regions mediating reinstatement of drug-seeking behavior such as the PFC, VTA, and amygdala [260]. Collectively, these studies suggest that endogenous KOR ligands mediate stress-induced reinstatement of self-administration and CPP and these effects are mediated by KOR regulation of monoamines.

Nicotine

Acute nicotine injection produces a dose-dependent increase in *pdyn* mRNA and DYN immunoreactivity in the striatum and NAcc [345]. PDYN elevations are long-lasting in striatum whereas peptide immunoreactivity is transiently increased (approx. 1 h). This is followed by a return to baseline and a rebound elevation of DYN content lasting up to 24 h. Like other drugs of abuse, systemic nicotine administration elevates extracellular NAcc DA levels [156]. Not surprisingly, D1 and NMDA receptor blockade attenuate nicotine-induced DYN elevations [345], again, highlighting the role of D1-NMDA interactions in modulating DYN synthesis in response to various drugs of abuse. Nicotine produces biphasic motivational effects across doses. Low to moderate doses produce CPP and high doses produce CPA [346, 347]. However, the neural mechanisms that mediate the aversive effects produced by acute administration of high-dose nicotine are not known. Low-dose nicotine administration does not alter the number DYN-positive neurons in the CeA or hypothalamus; nor were the number of dual-labeled DYN and C-FOS cells altered suggesting DYN neurons in these regions are not activated by low-dose nicotine administration [348]. Interestingly, doses (1–2 mg/kg; free base) that produce CPA also produce robust elevations of striatal DYN [345]. Thus, future experiments examining a role of KORs in mediating the aversive effects of high-dose nicotine are warranted. Repeated injections of low-dose nicotine decreases *pdyn* mRNA in the NAcc when administered three times a day [349], an effect not observed if the same nicotine dose is administered one time per day for 14 days [350]. This suggests a threshold of nicotine exposure required for long-term alterations in PDYN mRNA levels in the NAcc. *Pdyn* KO mice or wild-type mice treated with nor-BNI display similar nicotine-induced CPP [351, 352], suggesting that endogenous KOR signaling does not modulate the conditioned effects of nicotine. However, Galeote et al. [351] reported that acquisition of low-dose nicotine self-administration was enhanced in *pdyn* knock-out mice. These data may suggest that the activity of endogenous DYN/KOR systems serves an essential function in opposing the reinforcing effects of nicotine.

However, as with all studies using constitutive gene deletion, compensations that arise as a consequence of gene deletion may contribute to the observed phenotype. Unpublished observations indicate systemic U50,488-induced aversion and anxiety-like behavior are enhanced in nicotine-dependent animals relative to controls as assessed by CPA and the elevated plus-maze [353]. Moreover, the ability of systemic U50,488 to decrease NAcc DA overflow is exacerbated in nicotine-dependent adult rats relative to controls. These findings suggest that chronic nicotine exposure produces changes in KOR modulation of DA neurotransmission that lead to enhanced negative affect and increased anxiogenic effects. Alterations in KOR systems produced by chronic nicotine exposure play an important role in mediating nicotine withdrawal since nor-BNI pretreatment blocks somatic signs of spontaneous withdrawal in nicotine-dependent rats. Conversely, U50,488 administration potentiates somatic withdrawal signs. In agreement with these observations, Jackson et al. [352] reported that spontaneous nicotine withdrawal-induced anxiety-like behavior in the elevated plus-maze and somatic signs of withdrawal are blocked by nor-BNI or JDTic pretreatment. Moreover, these antagonists block expression of mecamylamine-precipitated nicotine withdrawal. Collectively, these results suggest that DYN/KOR alterations produced by chronic nicotine enhance KOR signaling during nicotine withdrawal that may result in dysphoria and physical signs of withdrawal. However, it should be noted that *pdyn* knock-out mice display similar mecamylamine-precipitated nicotine withdrawal signs as wild-types [351].

Ethanol

In vivo microdialysis demonstrated that acute administration of ethanol produces transient elevations in extracellular DYN A 1–8 in the NAcc [354]. Prolonged elevations in DYN A 1–8 are observed in the CeA at higher doses (2.0–2.8 g/kg) [355]. Chronic ethanol treatment decreases PDYN and DYN content in the hippocampus [356, 357], and chronic ethanol consumption increases PDYN in the hypothalamus, including the paraventricular nucleus [358, 359]. Voluntary ethanol consumption also produces elevations in CeA, and mPFC [359]. NAcc *pdyn* mRNA expression is unchanged [349, 359] or enhanced during withdrawal from repeated ethanol injection or consumption in rodents relative to controls [360, 361]. These discrepant findings may be explained by differences in exposure (i.e., passive injection or vapor vs. voluntary consumption in home cage). Alternatively, changes in DYN/KOR systems are likely dynamic with low levels of ethanol consumption acutely engaging DYN/KOR systems reward-related structures (i.e., NAcc) and larger, prolonged consumption recruiting DYN/KOR systems involved in drug craving and stress (i.e., CeA). It is expected that these dynamic changes will also differ depending the mode of ethanol exposure. Acute ethanol exposure enhances NAcc DA release more robustly in *orpk1* knock-out mice and in nor-BNI-treated wild-type mice relative to controls [362]. These findings suggest endogenous KOR systems function to decrease mesolimbic responsiveness to ethanol. Ten minutes U50,488 pretreatment prior to ethanol decreases ethanol-induced CPP using conditioning procedures under which U50,488 does not produce CPA suggesting U50,488 decreases the conditioned effects of ethanol independently of its aversive effects. Lindholm et al. [363] demonstrated that tonic inhibition of DA overflow by KORs in the NAcc is enhanced after repeated injections of moderate doses of ethanol, which may contribute to decreased motivation and negative affect after repeated ethanol exposure.

Findings regarding the effects of KOR ligands on ethanol self-administration are discrepant. Synthetic KOR agonists decrease ethanol consumption in limited-access, two-bottle choice procedures [364, 365]. Nor-BNI treatment enhances voluntary ethanol consumption in a continuous two-bottle choice paradigm. This effect occurs in rats with high, but not low, levels of ethanol consumption [366], suggesting that ethanol exposure elevates DYN tone in high drinkers. Nor-BNI pretreatment (24 h) does not alter ethanol self-administration but increases the latency for ethanol-induced NAcc DA overflow [367]. The mechanism mediating the delayed NAcc DA response is not known. However, 24 h nor-BNI pretreatment increases DA release and reuptake in mice [153], which may account for delayed mesolimbic DA responses in nor-BNI-treated rats self-administering ethanol. Overexpression of *bdnf* globally decreases ethanol consumption and this effect is by blocked by nor-BNI pretreatment [368], suggesting that DYN/KOR systems are a downstream effector of BDNF. Hypothalamic DYN/KOR systems have been implicated in ethanol-seeking behavior. Microinjection of U50,488 into the paraventricular nucleus of the hypothalamus decrease ethanol consumption in rats [369]. Mediodorsal hypothalamic DYN-positive neurons projecting to the paraventricular nucleus of the thalamus are activated after context-induced reinstatement of alcoholic beer self-administration and microinjection of U50,488 into this region decrease reinstatement in rats [370]. These studies suggest that hypothalamic DYN/KOR systems modulate ethanol-seeking behavior. However, it is not clear if these systems regulate ethanol consumption by modulating hypothalamic regulation of caloric intake or motivational processes associated with ethanol-seeking.

One hypothesis posits that ethanol-induced alterations in the DYN/KOR system in addiction circuitry increase negative reinforcement due to increased modulation of

drug-seeking neuronal substrates by DYN/KOR systems. Systemic and intracerebroventricular administration of nor-BNI blocks alcohol self-administration after abstinence in ethanol-dependent rats exposed to chronic ethanol vapors, an effect not observed in non-dependent rats [371, 372]. However, another study failed to find any effect of nor-BNI on abstinence-induced ethanol consumption in rats with prolonged ethanol experience [373]. Interestingly, acute and chronic administration of CI-977 enhanced both basal and abstinence-induced ethanol drinking at low doses that were ineffective in modulating ethanol and water intake [373]. These studies suggest that during ethanol abstinence in dependent animals, increased DYN tone in regions mediating craving, withdrawal, and/or stress reactivity produces negative reinforcing effects and this effect is mimicked by an exogenous KOR agonist. However, it is currently not clear whether nor-BNI attenuates alcohol-seeking by diminishing the negative affective properties of withdrawal and/or decreasing stress-induced alcohol self-administration. Taken together, these studies suggest that DYN/KOR systems in different regions are recruited with different time courses after acute and repeated ethanol exposure. This time and region dependency may explain some of the discrepancies observed between studies. For instance, KOR agonists may decrease ethanol-seeking behavior by decreasing DA and glutamate transmission in the NAcc. DYN/KOR systems in stress-related systems (i.e., CeA or bed nucleus of stria terminalis) may increase withdrawal-associated dysphoria during abstinence that promotes ethanol-seeking behavior. Work examining the role of DYN/KOR systems in mediating ethanol-seeking behavior motivated by different mechanisms (i.e., positive reinforcement vs. negative reinforcement) will further our understanding of this system in ethanol addiction. However, it has been difficult to determine the role of DYN/KOR systems during different phases of ethanol-seeking behavior due to long-lasting effects of KOR antagonists and the lack of sensitive methods that permit quantification of DYN release in vivo.

The role of KOR signaling in mediating stress-induced reinstatement of drug-seeking was recently extended to include ethanol. Stress-induced enhancement of ethanol self-administration and reinstatement of CPP is absent in *pdyn* knock-out mice and in wild-type mice treated with nor-BNI, suggesting that stress releases DYN which mediates stress-induced potentiation of the conditioned effects of ethanol and ethanol intake [374]. Additionally, administration of U50,488 mimicked the stress-induced enhancement of ethanol CPP and oral intake. Interestingly, Matsuzawa et al. [375], reported that U50,488 and nor-BNI administration attenuated and enhanced stress-potentiated ethanol CPP in rats, respectively. Importantly, the aversive effects of U50,488 were enhanced in stress versus non-stressed rats highlighting the complexity of the interaction of stress-related DYN signaling and its role in potentiation of CPP produced by ethanol and drug of abuse.

Opioids

Like other drugs of abuse, opiate treatment has been shown to alter DYN/KOR systems in reward-related neural circuits in animal models [376]. Chronic opiate administration also increases DYN A and B immunoreactivity in the NAcc, hypothalamus, and hippocampus of rats [376–379]. Chronic morphine increases *pdyn* expression in the LC of mice and rats [380], which is of interest since the LC has been implicated in mediating several behavioral effects of opiates [381]. Both systemic and intra-SN morphine administration increases SN extracellular DYN B levels, while systemic, intra-SN, and intra-striatal morphine perfusion is without effect on striatal DYN B overflow [382]. Given that a large proportion of DYN inputs to the SN arise from the dorsal striatum [189], activation of MORs in the SN may disinhibit DA neurons [383, 384]. This would result in positive modulation of the direct pathway of the basal ganglia, which would enhance SN extracellular DYN levels. DYN/KOR systems have been implicated in mediating the enhanced sensitivity to the reinforcing effects of opiates and other drugs of abuse in rodents. For instance, DBA/2J mice, which have a low drug responsivity, have greater *pdyn* mRNA expression in the NAcc core relative to drug responsive C57BL/6J mice [385]. Interestingly, nor-BNI facilitates sub-threshold morphine CPP in DBA/2J, but not C57BL/6J, mice, suggesting that increased NAcc DYN/KOR tone in these mice decreases morphine reward in DBA/2J mice.

KOR agonists decrease opiate self-administration maintained under limited access [328, 386], an effect associated with decreased NAcc DA levels [386]. Dorsal striatal DYN A- and B-immunoreactivity tissue content is enhanced in heroin-experienced rats when heroin access is expected but not immediately after extended heroin self-administration, an effect not observed with cocaine [387]. This suggests that DYN/KOR systems may be altered by expectancy of drug availability rather than by direct opiate exposure and these changes may result in opiate craving. However, nor-BNI does not alter heroin or morphine self-administration under limited access conditions [328, 386, 388]. Interestingly, nor-BNI enhances the increase in NAcc DA release that occurs during heroin self-administration [386], suggesting that endogenous NAcc KOR ligands may inhibit mesolimbic DA neurotransmission without altering heroin self-administration maintained under fixed ratio responding. It is of importance to determine whether endogenous DYN/KOR systems modulate withdrawal. A

recent study reported that the KOR antagonist, GNTI, failed to modify withdrawal-induced choice of heroin over food in non-human primates with 21-h access to heroin [389], suggesting KORs may not regulate opiate withdrawal-induced heroin seeking. Future research aimed at determining the role of KOR systems in modulating opiate-withdrawal-induced drug-seeking and whether these processes are altered in animals with an extended history of opiate use, or stress, and in preclinical models of psychiatric disorders is needed.

Unlike other drugs of abuse, most studies have reported that KOR activation by endogenous or exogenous ligands decreases the severity of opiate withdrawal. Systemic and central administration of nor-BNI increases somatic signs of opiate withdrawal and the conditioned effects of morphine withdrawal [390]. Moreover, naloxone-precipitated morphine withdrawal decreases DA overflow in the NAcc; an effect that is more robust in nor-BNI-treated rats relative to vehicle controls [390], suggesting that mesolimbic DA responses may be associated with enhanced expression of somatic and conditioned withdrawal. Intra-NAcc microinjection of nor-BNI increases somatic signs of morphine withdrawal [103]. Interestingly, intra-LC U50,488 administration blocks morphine-withdrawal-induced excitation of LC neurons, presumably by activating presynaptic LC neurons [204]. Nor-BNI treatment can also precipitate morphine withdrawal in dependent rats [391]. Given that nor-BNI may interact with MORs for some hours after its administration, nor-BNI may precipitate withdrawal by blocking MORs. However, another study reported that nor-BNI pretreatment does not produce somatic signs of withdrawal in morphine-dependent rats [392]. Conversely, somatic signs of morphine withdrawal are decreased in *oprk1* knock-out mice [393] and to a lesser extent in rats treated with JDTic, suggesting that DYN/KOR systems may contribute to somatic signs of withdrawal [394].

Therapeutic implications

Careful considerations must be made when deciding how KOR ligands should be used as therapeutic targets since the DYN/KOR system undergoes dynamic changes during various stages of the addiction cycle. Studies suggest that endogenous DYN/KOR systems are recruited in response to acute exposure to drugs of abuse and this counteracts reward/approach behaviors. As such, KOR agonists would be expected to initially decrease the rewarding effects of drugs of abuse and, perhaps, deter the development of addictive behavior. Chronic exposure and drug-seeking behavior alter the activity of the DYN/KOR system promoting negative affect and heightened vulnerability to stress during withdrawal from drugs of abuse. Thus, antagonism of KOR may ameliorate negative reinforcing behavioral states associated with drug withdrawal (e.g., nicotine or ethanol). In light of research demonstrating that KORs are powerful effectors of behavioral stress responses, KOR antagonists may be effective in ameliorating stress-induced drug-seeking behavior. Future research examining interactions between drug dependence and KOR modulation of stress responses are needed since stress systems are altered after prolonged drug exposure and contribute to drug addiction [291]. Questions remain regarding the interaction of KOR antagonists with chronic, rather than acute stress and whether antagonists would be beneficial in individuals in whom stress may be unpredictable. That is, are KOR antagonists effective if administered after the onset of stress? Importantly, conditioned stimuli associated with drug use precipitate relapse to addiction. To date, studies assessing the influence of KOR ligands on drug-seeking maintained by such stimuli are limited. Finally, increasing data indicate that with more prolonged drug experience, drug seeking that is initially goal-oriented becomes habitual [395]. Most pre-clinical studies have assessed the influence of KOR ligands during limited cocaine access, using reinforcement schedules that may not result in habitual responding. The recent description of a rodent model which allows delineation of goal-oriented versus habitual intravenous drug administration will now allow direct assessment of the role of KOR systems in these stages of addiction [396].

DYN/KOR system in schizophrenia

Schizophrenia is a disorder with strong neurodevelopmental, environmental, and polygenetic components. It is characterized by three major symptom clusters [397]: (1) positive symptoms involving auditory and to a lesser extent visual hallucinations, feelings of grandeur, delusional behavior, and thought disorder; (2) negative symptoms, which include social withdrawal, blunted affect/mood, decreased motivation, poverty of speech, and anhedonia (inability to experience pleasure); (3) cognitive deficits which include deficits in executive function, working memory, and attentional processes. Typically, schizophrenia is diagnosed during late adolescence or early adulthood in men after their first psychotic break, although negative symptoms and cognitive deficits may present prior to psychosis onset. It is believed that positive symptoms are mediated by enhanced DA transmission in the ventral striatum as they correlate with DA binding to D2 receptors [398]. Therapeutic efficacy is variable between patients and symptom cluster-dependent, with typical and atypical antipsychotics having some therapeutic value for positive symptoms [399]. However, effective therapeutics for

negative symptoms or cognitive deficits are, to date, lacking since the underlying mechanisms are not understood, although they are thought to involve dysfunction of GABA and glutamate neurotransmission [400].

The DYN/KOR system has been implicated in schizophrenia in light of research demonstrating that a synthetic KOR agonist produce psychomimetic effects in humans, including hallucinations, perceptual distortions, and depersonalization [229]. Recently, converging results demonstrating that salvinorin A, a psychoactive compound in the hallucinogenic plant *S. divinorum*, is a potent KOR agonist have revived interest in the role of the DYN/KOR system in schizophrenia [401]. However, it should be noted that the effects of salvinorin A have not been examined in patients with schizophrenia, and salvinorin A may produce differential behavioral effects in this population.

CSF and tissue levels of DYN in schizophrenia

In the 1980s, several groups examined whether DYN peptide levels in spinal CSF were altered in schizophrenic patients. However, disparate findings were obtained. Heikkila et al. [402] reported enhanced CSF DYN A levels in unmedicated patients with schizophrenia relative to healthy and psychiatric controls using an antibody recognizing DYN A (9–17). CSF DYN A levels significantly correlated with increased psychotic rating scores on the Brief Psychiatric Ratings Scale. Increased CSF levels of DYN A have been reported in patients with schizophrenia with a poor prognosis; whereas patients with schizophrenia with a good prognosis have similar DYN A CSF levels relative to healthy controls [403]. Although correlative, these studies suggest that elevated endogenous DYN levels may contribute to the psychopathology of schizophrenia. However, Zhang et al. [404] reported lower DYN A (1–8) immunoreactivity in CSF from schizophrenia patients relative to controls with neurological disorders. The lack of healthy controls in that study makes interpretation of the findings difficult since DYN/KOR system dysregulation has been implicated in several neurological disorders. It is currently not clear to what extent spinal CSF DYN levels reflect alterations in limbic and cortical DYN activity since abnormalities in these systems has been implicated in the pathophysiology of schizophrenia. Nonetheless, these pioneering studies provided suggestive evidence that central DYN content may be altered in patients with schizophrenia. Analysis of *PDYN* and *OPRK1* expression and KOR binding in post-mortem tissue has also yielded inconsistent results. *PDYN* and *OPRK1* mRNA levels are unchanged in dorsal lateral prefrontal and cingulate cortex as well as in the amygdaloid nuclei of schizophrenics relative to controls [280, 281]. Fourier analysis of laminar KOR immunoreactivity in the hippocampus of patients with schizophrenia revealed that, unlike controls, the former group did not show a consistent laminar distribution of KORs [405]. It is possible that the abnormal KOR distribution observed is due to general disorganization of hippocampal circuits in schizophrenia rather than to altered KOR distribution. Moreover, sample size in this study was small.

Discriminative stimulus effects of KOR agonists

KOR agonists produce psychomimetic effects similar to ketamine and PCP. The latter drugs induce psychomimesis in healthy humans and symptoms in symptom-free patients with schizophrenia [406]. However, in non-human primates, the discriminative stimulus effects produced by salvinorin A do not generalize to those produced by ketamine or the 5-HT receptor agonist, psilocybin [407, 408]. Furthermore, Killinger et al. [409] demonstrated that the discriminative stimulus effects of salvinorin A do not generalize to LSD, another 5-HT receptor agonist. Others have reported that non-competitive NMDA receptor antagonists (ketamine, PCP, MK-801), but not competitive antagonists, generalize to the discriminative stimulus effects of U50,488, but not the KOR agonist TRK-820 [410]. Collectively, these findings suggest that psychomimetic effects produced by salvinorin A differ from those produced by classical hallucinogens. Given that PCP and ketamine produce psychomimetic effects that are qualitatively similar to sensory hallucinations and perceptual distortions in schizophrenia and KOR-agonists-induced discriminative stimulus effects may differ from PCP and ketamine, alterations in DYN/KOR function in schizophrenia appear insufficient to fully account for abnormal perception.

DYN/KORs and prepulse inhibition

Prepulse inhibition (PPI) is a sensory-gating process where a weak stimulus preceding a robust stimulus will diminish the behavior associated with the strong stimulus. PPI deficits are present in patients with schizophrenia and unaffected siblings [411]. Bortolato et al. [412] demonstrated that U50,488 disrupted PPI of the acoustic startle in rats, an effect reversed by atypical, but not typical, antipsychotic pretreatment. However, using various synthetic KOR agonists and salvinorin A, we found no effect of KOR activation on PPI [413]. Furthermore, KOR blockade did not alter basal PPI levels. Stress exacerbates or triggers symptoms in patients with schizophrenia [414]. Central CRF infusions decrease PPI, providing a framework by which stress could disrupt PPI in schizophrenics. CRF-induced PPI deficits were not altered in nor-BNI-treated rats, suggesting that endogenous DYN/KOR signaling is not necessary for basal PPI and is not a downstream mediator of CRF-induce PPI deficits. This contrasts with

the downstream role of DYN/KOR in mediating stress-related anxiety and drug-seeking behavior, suggesting that CRF/KOR system interactions may be limited to certain behaviors, including affective and reward-related behaviors.

Co-morbidity of drug abuse and schizophrenia

The majority of patients with schizophrenia have co-morbid substance use disorders [397]. Given that alterations in the DYN/KOR in cortical and limbic regions implicated in schizophrenia have been reported in animal models of drug addiction and in dependent/addicted humans, it is possible that alterations that may be present in DYN/KOR systems in patients with schizophrenia stem from substance abuse. Alternatively, drugs of abuse may have differential effects on DYN synthesis and release in patients with schizophrenia or in animal models of schizophrenia relative to controls. For example, burst stimulation of the VTA results in a DA-dependent, sustained depolarization of medium-sized spiny NAcc neurons and decreased MSN firing in vivo in control rats [415]. Conversely, in neonatal ventral hippocampal lesion (NVHL) rats, a neurodevelopmental animal model of schizophrenia, VTA stimulation increases NAcc MSN activity [415]. Given that NAcc D1 DA receptor activation enhances PDYN synthesis, DYN may be synthesized in response to behaviors associated with enhanced NAcc DA release (i.e., drugs of abuse; stress) in controls, but only released in response to strong cortical or limbic drive of DYN-containing NAcc MSN neurons. In NVHL rats, drug-seeking behavior would result in exacerbated MSN activity and subsequent DYN release, resulting in enhanced DYN tone. This is consistent with elevated levels of *pdyn* expression in the striatum of NVHL during development [416], and during adulthood [417]. Unfortunately, to date this hypothesis has not been tested. An understanding of DYN/KOR dynamics in striatal, cortical, and limbic regions in animal models of schizophrenia will further our understanding of the role of this system in the pathophysiology of schizophrenia since it is hard to control for effects of drug abuse, stress, and antipsychotic medication in patients, all of which are associated with DYN/KOR system activation [151, 184, 279].

DYN/KOR systems in cognition

Deficits in cognitive function are observed in drug addiction, schizophrenia, and depression [418–420]. The DYN/KOR system modulates cognitive processes. This may result from control of neurotransmission by DYN/KOR systems in brain regions that mediate working memory and decision-making.

KORs and PFC-dependent processes

Systemic administration of KOR agonists decreases performance on the five-choice serial reaction time task (5-CSRTT), a task that assesses attentional processes and impulsive behavior that are dependent on appropriate PFC function [421–423]. Typically, decreased correct responses are observed after systemic KOR agonist administration, indicating impaired attentional processes. However, systemic KOR agonists also increase latency to respond and the number of trials that are omitted in the 5-CSRTT, which could reflect the influence of KOR ligands on motivation. This latter effect is a potential confound in interpreting data from cognitive tasks that require food as a motivator. Nemeth et al. [421] compared the effects of reward satiety, which decreases motivation to obtain reward, and systemic salvinorin A in the 5-CSRTT paradigm. Indeed, both reward satiety and salvinorin A decrease latency to response and trial omission indicative of decreased motivation. However, salvinorin A, but not reward satiety, decreases correct responding in the 5-CSRTT, suggesting that KOR agonism may exert detrimental effects on attentional processes independently of effects on motivation.

KOR and hippocampal-dependent cognition

Microinjections of DYN A 1–8 into CA3 of dorsal hippocampus produce a naloxone-reversible decrement of performance in tasks of spatial working memory [424]. In contrast, passive avoidance behavior is unaltered. Intrahippocampal microinjection of DYN B or U50,488, decrease spatial working memory as assessed by the Morris water maze and this effect is reversed by nor-BNI [425, 426], suggesting that activation of CA3 hippocampal KOR modulates spatial memory. KOR blockade does not alter spatial memory [425, 426], indicating that KOR does not tonically inhibit hippocampal-dependent working memory. Consistent with pharmacological approaches, *orpk1* knockout mice do not display alterations in spatial working memory as assessed by the eight-arm radial maze and Morris water maze [427]. However, it is difficult to observe improvements in most cognitive tasks except under conditions where cognitive load is increased. Activation of the DYN/KOR system negatively modulates hippocampal synaptic plasticity. LTP has been suggested to be one type of synaptic plasticity that mediates learning and memory function [428]. As mentioned above, KOR activation decreases neurotransmission of both perforant path and mossy fiber pathways in the hippocampus [176, 223, 224]; providing a cellular basis for the detrimental effect of DYN and exogenous KOR agonists on hippocampal-dependent spatial memory.

Stress has been reported to exert both beneficial and detrimental effects on cognition. Recently, Carey et al. [429] demonstrated that the disruptive effects of forced swim stress on performance in the novel object recognition task is blocked by nor-BNI pretreatment in wild-type mice and absent in *pdyn* knock-out mice. Additionally, U50,488 administration decreases performance of wild-type mice in the novel object recognition task. Acute stress and CRF increase DYN synthesis and KOR phosphorylation [261, 266, 283], thereby, inhibiting glutamatergic signaling in brain regions (e.g., hippocampus, frontal and temporal cortex) that mediate cognitive function (e.g., working memory, attention, decision-making). Future research should be aimed at determining whether the modulatory role of the DYN/KOR system on cognition is altered in animals with repeated stress, a history of drug exposure, or in animal models of schizophrenia; all conditions in which DYN/KOR systems are altered.

DYN and age-related cognitive decline

Dysregulation of the DYN/KOR system is implicated in cognitive decline associated with aging. Enhanced PDYN and DYN peptide levels have been reported in the frontal cortex and hippocampus of aged rats (25–27 months of age) [430, 431]. Increased PDYN and DYN 1–8 content in the hippocampus and frontal cortex is negatively associated with performance on the Morris water maze, a spatial memory task highly dependent on proper hippocampal function [431]. Interestingly, aged rats that performed well during testing had DYN levels comparable to that of young adult controls. In partial agreement with these observations, Nguyen et al. [432] reported a small amelioration of deficits in Morris water maze performance in aged *pdyn* knock-out mice relative to aged wild-type controls. However, in the latter study, mice were 13–17 months of age and did not show elevated hippocampal DYN 1–8 peptide levels. Thus, the small effect size may be attributed to the use of younger animals that do not have altered DYN levels. The effects of KOR blockade on age-related cognitive effects have not been examined. However, enhanced DYN tone in the aged hippocampus may contribute to cognitive decline by decreasing hippocampal glutamatergic transmission via KOR activation or through DYN actions on non-opioid receptors.

DYN/KOR system in Alzheimer's disease

Changes in KOR have been observed in the striatum of Alzheimer's patients but the data are inconsistent, with decreased [433], increased [434, 435], or no difference [436] in KOR binding relative to controls. Enhanced amygdala KOR binding is present in Alzheimer's patients [433] whereas several studies reported no differences in KOR binding in frontal and temporal cortex [433–435]. Interestingly, increased DYN A 1–8, but not DYN B, immunoreactivity is observed in frontal cortical tissue of patients with Down syndrome and Alzheimer's disease [437]. Furthermore, DYN A 9–17 is elevated in cortex of Alzheimer's patients relative to typical, Parkinson's disease, and cerebrovascular disease controls, an effect not observed for PDYN and DYN B [438]. Given the existence of tonically active KORs in the mPFC that inhibit DA overflow, these findings raise the possibility that enhanced PFC DYN A tone may alter mPFC DA dynamics in Alzheimer's disease and Down syndrome since DA signaling is critical for optimal PFC function (e.g., working memory and behavioral flexibility) [199, 439].

Beneficial effects of DYN in models of cognitive impairment

Contrary to findings that DYN/KOR activation impairs cognitive processes, a number of studies have demonstrated that DYN and synthetic KOR agonists ameliorate cognitive deficits in animal models of cognitive impairment. The detrimental effects of nicotinic acetylcholine receptor (nAChR) and muscarinic acetylcholine receptor (mAChR) ligands are reversed by DYN peptides and U50,488 in a nor-BNI-sensitive manner [440, 441]. However, it should be noted that nor-BNI was administered intracerebroventricularly 5 min prior to KOR ligand administration. Consistent with nor-BNI-sensitive effects of KOR ligands on cognitive impairment, improvement of mAChR-antagonism-induced cognitive impairment by U50,488 is blocked by antisense oligonucleotides targeting KOR [442], suggesting that KOR activation is necessary for this effect. DYN (2–13), a PDYN-derived peptide that does not interact with opioid receptors, also blocks cognitive impairments induced by mAChR ligands, suggesting that non-opioid actions of DYN peptides may contribute to this effect.

PDYN and *OPRK1* in the genetics of psychiatric disorders

Heritability of psychiatric disorders is estimated to be substantial (e.g., 73–90% in schizophrenia [443]). Although the bulk of variance is yet to be explained [444, 445], multiple polymorphisms in human *PDYN* and *OPRK1* have been associated with drug addiction or schizophrenia [446, 447]. We will limit our discussion to variants for which there is evidence of function (overviewed above). The frequency of schizophrenia is higher in individuals with three copies of rs35286281 and who are also homozygous for the C

(glycine) allele of rs6280 (SNP) located in exon 2 of the DA D3 receptor gene, *DRD3* [448]; mutation which confers higher DA affinity and altered signaling in vitro [449, 450]. Given the likely polygenic mode of inheritance of schizophrenia [451], and research demonstrating that the DYN/KOR system and D3 receptors regulate DA release and reuptake [185, 452, 453], this apparent gene–gene interaction suggest that concomitant dysregulation of these systems may contribute to altered DA transmission during psychosis. More specifically, altered KOR- and D3 receptor-mediated control of DA dynamics in the NAcc (where there is a high overlap between DYNs, KORs, and D3 receptors) may result in increased presynaptic control of DA efflux during development.

Although the results are divergent, rs35286281 and rs1997794 have been linked to psychiatric disorders on a number of occasions [69, 454, 455]. In some cases, however, data interpretation may have been confounded by population stratification (the presence in study samples of individuals from different population subdivisions with putatively different allele frequencies). rs910080 has been associated with alcohol dependence and episodic memory in the elderly [455, 456]. rs35566036 was associated with alcohol dependence in a family-based study [73], adding to the number of SNPs in *OPRK1* associated with this disorder [455]. It should be noted that rs35566036 was not included in a linkage study on a genetically more heterogeneous sample which yielded seemingly conflicting results to those of Xuei et al. [457]. rs6985606 and rs997917 (SNPs) located in intron 2 of *OPRK1*, however, were associated with alcohol dependence in both studies when the variance introduced by age and gender was accounted for by Zhang et al. ($p \leq 0.05$). The haplotypes (alleles on the same chromosome in linkage disequilibrium at two or more loci) associated with alcohol dependence by Zhang and colleagues were not inferred by Xuei et al. Thus, these studies are in concordance except with respect to the haplotype blocks identified, which differ at one position.

The linkage studies on *PDYN* and *OPRK1* performed to date have been targeted to test specific hypotheses (i.e. validating previous findings) permitting a significance threshold of $p < 0.05$. However, genome-wide significance (i.e., $p < 10^{-4}$) has not been reported for either gene; a discrepancy potentially explained by insufficient power still in genome-wide association studies (GWASes) of psychiatric disorders [444]. Power can be increased by enlarging the sample size and/or refining the phenotype. In this regard, it is interesting to note that a region on chromosome 20 comprising *PDYN* was recently linked to heavy alcohol consumption in a GWAS on a comparatively large sample [458], and that nominal support was found for this gene in a family-based GWAS of alcohol dependence [459]. Accurate estimates of the genotype relative risks inferred by the aforementioned SNPs in *PDYN* and *OPRK1*, however, likely awaits analyses of larger samples and/or meta-analyses.

First-generation GWASes were designed to test the common disease common variant hypothesis according to which psychiatric disorders are caused by multiple common variants (frequency of >5%) with small effect sizes (e.g., the SNPs in *PDYN* and *OPRK1* mentioned herein) [460]. They are based on linkage disequilibrium bins (i.e., groups of highly correlated SNPs each represented by a tagging SNP) [461]. These tagging SNPs capture some 40–50% of common structural variants [sequence variants other than SNPs (e.g., rs35286281 and rs35566036)] [462]. This is a noteworthy limitation given that such variants account for an estimated 74–92% of all variant bases. Moreover, they do not capture rare variants (frequency of <1%) [463]. Thus, that only a small percentage of the genetic variance in psychiatric disorders has been explained by the GWASes performed to date is not surprising [444]. The combined results of these studies suggest that the low genotype relative risks inferred by common SNPs preclude them from being causative of the familial clustering of most psychiatric disorders [445].

Contrary to the common disease common variant hypothesis, the multiple rare variant hypothesis predicts that psychiatric disorders are the result of multiple rare, mainly non-synonymous (coding) variants with comparatively large effect sizes [464]. In addition to the apparent failure of GWASes to securely implicate specific genes in common disease [465], support for this hypothesis comes from monozygotic/dizygotic twin concordance ratios for some psychiatric disorders [e.g., drug (other than cocaine) addiction] [451]. Rare missense mutations in the coding region of *PDYN* were recently reported to cause the late-onset neurodegenerative disorder spinocerebellar ataxia type 23 [466]. Thus, it would be interesting to assess the prevalence of these mutations in families with multiple affected members and/or in cohorts that are at the extreme ends of psychiatric disorders. However, neither hypothesis alone is likely to explain all of the genetic variance in psychiatric disorders [460], and a complete understanding of the etiologies of psychiatric disorders in *cis* may await development of methods to accurately, cost-effectively and rapidly re-sequence entire genomes. Clues to the apparent shortcomings of GWASes may also come from ever more comprehensive epigenomic analyses as suggested by the identification of intergenic, often differentially methylated regions in the GWASes of disease performed to date [467].

Conclusions

DYN/KOR systems are endogenous, activity-dependent modulators of neuronal circuits that mediate affect,

motivation, stress reactivity, and reward. DYN/KOR systems are recruited by various stimuli and act to shape neuronal activity, alter presynaptic neurotransmitter release, and decrease neuronal excitability. Dysregulation of neuronal activity in cortical-limbic-basal ganglia circuitry is believed to underlie behavioral abnormalities that are commonly shared by psychiatric disorders. Aberrant neuronal activity in cortical and limbic regions as well as in the basal ganglia produces long-lasting changes in DYN/KOR systems. Changes in this system may contribute to symptom clusters that are shared by various psychiatric disorders (i.e., decreased motivation and negative affect). A role for DYN/KOR in modulating drug addiction has been proposed. However, with improved research techniques and animal models of addiction it is now appreciated that the function of DYN/KOR systems in addiction is diverse and that this system may bi-directionally modulate drug-seeking behavior depending on drug history, pattern of intake, incentive salience, and stress. Future research is warranted to determine whether genetic predisposition and/or environmental factors may dictate therapeutic utility of KOR ligands. Similarly, there is compelling evidence from preclinical studies that the DYN/KOR system may dysregulated in affective psychiatric disorders. However, solid evidence from clinical studies is lacking. Thus, with the advent of new research tools (i.e., radiolabeled KOR ligands), work aimed at determining the physiological and pathophysiological role of DYN/KOR systems will further our understanding of this system in psychiatric disease in humans. Moreover, translational studies may provide insight to the mechanisms by which DYN/KOR contribute to brain dysfunction during the course of a disease and at what stage of the disease process, drugs that target this opioid system may have therapeutic effects.

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