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Neural circuit mechanisms of the cholecystokinin (CCK) neuropeptide system in addiction

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Abstract

Given historical focus on the roles for cholecystokinin (CCK) as a peripheral hormone controlling gastrointestinal processes and a brainstem peptide regulating food intake, the study of CCK as a limbic neuromodulator coordinating reward-seeking and emotional behavior remains underappreciated. Furthermore, localization of CCK to specialized interneurons throughout the hippocampus and cortex relegated CCK to being examined primarily as a static cell type marker rather than a dynamic functional neuromodulator. Yet, over three decades of literature have been generated by efforts to delineate the central mechanisms of addiction-related behaviors mediated by the CCK system across the striatum, amygdala, hypothalamus, and midbrain. Here, we cover fundamental findings that implicate CCK neuron activity and CCK receptor signaling in modulating drug intake and drug-seeking (focusing on psychostimulants, opioids, and alcohol). In doing so, we highlight the few studies that indicate sex differences in CCK expression and corresponding drug effects, emphasizing the importance of examining hormonal influences and sex as a biological variable in translating basic science discoveries to effective treatments for substance use disorders in human patients. Finally, we point toward understudied subcortical sources of endogenous CCK and describe how continued neurotechnology advancements can be leveraged to modernize understanding of the neural circuit mechanisms underlying CCK release and signaling in addiction-relevant behaviors.

Keywords

Cholecystokinin; Neuropeptide; Circuit; Addiction; Alcohol; Reward

Introduction

Cholecystokinin (CCK) is the most abundant neuropeptide, widely distributed in the brain and central nervous system [51]. CCK is also localized to peripheral sites including the extraintestinal endocrine cells and cardiomyocytes. First discovered in the gastrointestinal system, the role of CCK in digestion has been well-explored. However, the exact role of

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CCK in the central nervous system remains elusive. There has been a continued interest to decipher how CCK-expressing interneurons contribute to hippocampal and cortical circuits [44], however most studies examined CCK solely as a molecular marker rather than a functional peptide modulator [37]. In addition, beyond the cortex and hippocampus, dense clusters of CCK neurons exist throughout the amygdala, hypothalamus, mesolimbic dopamine (DA) system, and periaqueductal midbrain cell groups [5, 21, 34], indicating a broad diversity of mechanisms for CCK neuromodulation of emotional states (see Fig. 1).

The organization of CCK-releasing pathways in the brain remains understudied and largely relies on historical reports using classical lesion methods that pointed mostly toward ipsilateral long-range projections. For example, Zaborsky et al. and You et al. used radioimmunoassay and microdialysis to identify partial reductions of CCK protein levels in downstream forebrain target areas of the striatum and pallidum following either transection of the medial forebrain bundle, destruction of the piriform cortex, widespread cortical lesion, or 6-hydroxydopamine lesion of the ventral tegmental area (VTA) [80, 82]. These data indicate that extracellular CCK content in the nucleus accumbens (NAcc), ventral pallidum (VP), and bed nucleus of the stria terminalis (BNST) originates from the cortex and mesolimbic DA system, but also from additional unidentified central sources.

Like many other neuropeptides, CCK co-localizes and interacts with other neurotransmitters and neuromodulators, such as DA, gamma-aminobutyric acid (GABA), serotonin (5HT), and endogenous opioid peptides [51]. It is possible that CCK serves as an intercellular messenger peptide locally and/or distally to represent internal physiological state and emotional state. Indeed, activation of CCK receptors has been shown to modulate the mesolimbic reward system, anxiety, and satiety [2]. These behavioral effects of CCK suggested its role in the neurobiological processes underlying addiction, and over the past 30 years, several studies directly tested the link between CCK and phenotypes relevant to the etiology of substance use disorders. However, many of these experiments relied solely on pharmacological methods and were performed nearly exclusively in male rats, limiting understanding of potential sex differences and circuit mechanisms in addiction-relevant CCK function. Recent advances in neurotechnology now allow detailed examination of CCK neuronal pathways and peptide signaling with unprecedented resolution, standing to substantially revise the framework for understanding CCK functions in reward-seeking behaviors. In this review, we summarize the evidence that supports the role of CCK in behaviors related to addiction to psychostimulants, opioids, and alcohol, shedding light on future studies on CCK and potential therapeutic strategies for substance use disorders.

Molecular characterization of CCK

Biogenesis and degradation of CCK has been reviewed in [4, 11, 59]. CCK is synthesized and released in various molecular forms across species, with different cellular localization and affinity to receptors. Only one CCK mRNA has been identified, which codes for the 115 amino acids product preproCCK. Bioactive CCK peptides are derived from a 58 amino acid sequence. The C-terminal pentapeptide amide of CCK is critical for biological effects, and is also conserved across other homologous peptides such as gastrin. Neurons mainly release the C-terminal octapeptide CCK-8 and also CCK-5. Sulfation of the tyrosyl

residue in position seven determines CCK's receptor affinity. The sulfated CCK octapeptide (CCK-8S) is widely distributed in the central nervous system; however, the mesencephalon may contain substantial amounts of unsulfated CCK-8 [6]. Degradation of CCK may involve membrane-bound aminopeptidases, a membrane-bound isoform of tripeptidyl peptidase, and neprilysin (enkephalinase), a membrane-bound metalloendopeptidase that also inactivates other peptides such as enkephalin and angiotensin [15, 60, 84].

CCK receptors

CCK peptides act through two G-protein coupled receptor subtypes, CCK-A (CCK1) receptor, and CCK-B (CCK2) receptor, encoded by the genes *Cckar* and *Cckbr*, respectively. CCK1 receptor is highly expressed in the gastrointestinal tract, binding to CCK-8S with high affinity. CCK2 receptor is the primary subtype in the brain and also in the stomach, binding to non-sulfated and sulfated CCK-8, gastrins, and C-terminus fragments such as CCK-5. Although expressed at lower levels than CCK2R, CCK1R in the central and peripheral system mediates satiation signals and other behaviors, previously reviewed in detail [2]. It is worth noting that like some other GPCRs, CCK1R and CCK2R are capable of coupling with multiple G-protein subtypes. CCK1R predominately couples with Gq, but also Gs, Gi, and G13; CCK2R functions through Gq and also Gi signaling [45, 83].

Regarding presynaptic vs. postsynaptic expression patterns of CCK receptors, little is known within the classical addiction neurocircuits. CCK1R appears to be located presynaptically on habenular inputs to the interpeduncular nucleus [33], as well as in the parabrachial nucleus and area postrema where it presynaptically modulates excitatory transmission [65, 68]. Other reports identified a postsynaptic effect of CCK2R in layer 6b cortical neurons [10] and presynaptic actions of CCK2R in the hippocampus to facilitate glutamate release [4,7, 14].

CCK and psychostimulants

Discovery of abundant CCK co-localization with DA in neurons of the midbrain tegmentum initiated strong interest in studying the CCK system in DA-dependent effects of psychostimulant drugs of abuse, particularly with regard to midbrain DA projections to the ventral striatum, reviewed previously in detail [72] and summarized in Table 1. For example, amphetamine and methamphetamine administration increased CCK messenger ribonucleic acid (mRNA) levels throughout the mesolimbic DA pathway, though these effects may be particularly time-sensitive [36, 49]. Early findings also suggested an inverse relationship between levels of ventral midbrain CCK expression and DA neuron activity associated with locomotor activity and drug self-administration [48]. In line with these data, CCK2R pharmacological blockade or CCK1R genetic knockout in rats led to elevated baseline DA levels in the striatum and potentiated striatal DA release in response to cocaine or amphetamine administration [19, 46].

However, Vaccarino proposed that CCK could have both DA-enhancing and DA-limiting effects in the ventral striatum based on the degree of CCK release, presynaptic vs. postsynaptic locations of CCK1R vs. CCK2R, and the rostral-caudal sites of CCK activation in the nucleus accumbens (NAcc) [72]. Indeed, CCK1R receptor

blockade, either systemically or directly in the NAcc, decreased amphetamine-induced locomotor sensitization [16, 79], opposite to earlier findings with a CCK2 receptor antagonist [32]. Furthermore, intra-NAcc CCK2R agonist administration increased the progressive ratio breakpoint in amphetamine self-administration experiments, suggesting that NAcc CCK2R signaling has anti-reward effects [8]. These results are analogous to findings that intracerebroventricular (i.c.v.) administration of the CCK-8 peptide blocked methamphetamine-induced acute locomotor stimulation and progressive sensitization [26].

In a model of stress-induced reinstatement of cocaine-seeking behavior, Liu et al. found a protective effect of CCK2 receptor blockade [47], consistent with the suggestion by some that CCK2 receptor antagonists may have utility in treating cocaine addiction [13, 18]. Yet, we emphasize that nearly all the above studies were conducted in male rats. More recent findings that estradiol (E2) interacts with cocaine exposure to increase CCK protein levels in the ventral tegmental area (VTA) of ovariectomized female mice [54] suggest a critical examination of sex differences and hormonal influences on CCK's role in responding to psychostimulant drugs.

CCK and opioids

Based on literature implicating the CCK system in regulation of pain and anxiety, Hebb et al. hypothesized "anti-opioid" functions of CCK action throughout mesolimbic circuits [28]. Consistent with this idea, evidence from genetic knockout mice showed that loss of the CCK2 receptor resulted in upregulation of central endogenous opioid peptides [55]. Furthermore, Noble et al. [53] identified a relationship between higher expression of CCK/CCK receptor signaling and greater sensitivity of a CCK2R antagonist to promote opioid reward [53]. Behavioral effects and molecular changes resulting from altering CCK signaling activity in animal models of opioid abuse are summarized in Table 2.

While effects of CCK system manipulations on intravenous opioid self-administration were mixed [29], the reward-seeking model of conditioned place preference (CPP) demonstrated opposite actions of CCK1R and CCK2R antagonists in blocking and potentiating the acquisition of morphine CPP, respectively [30,31]. I.c.v. administration of the CCK-8 peptide blocked the acquisition and potentiated the expression of morphine-induced CPP, consistent with proposed anti-opioid effects of CCK [74]. However, in contrast to data supporting the anti-opioid hypothesis, experiments on aversive effects of opioid withdrawal indicated that CCK can have pro-opioid actions as well. For example, CCK-8 acted in a CCK1R-dependent manner to attenuate conditioned place aversion (CPA) and anxiety-like behavior resulting from naloxone-induced precipitation of morphine withdrawal [75, 81], and CCK restored morphine-induced disruption of hippocampal LTP [76].

Once more, it should be noted that the above studies were conducted nearly exclusively in male subjects. Data from ovariectomized female rats found that the opioid antagonist naltrexone potentiated E2-induced increases in CCK mRNA within the bed nucleus of stria terminalis (BNST) of the extended amygdala, indicating the potential for major sex differences and hormonal influences on opioid-CCK interactions in limbic circuits [17, 50].

CCK and alcohol

Given the unique caloric properties of ethanol, previous hypotheses highlighted the overlap in neuropeptide pathways that regulate both alcohol drinking as well as feeding, including CCK [70]. While i.p. injections of CCK decreased alcohol intake in rats, this was proposed to act through a peripheral mechanism, consistent with the well-documented role of brainstem and peripheral CCK actions in mediating satiety [38–43, 70]. Nevertheless, CCK1 and CCK2 receptor blockade reduced alcohol drinking and anxiety during alcohol withdrawal, respectively, hinting at unique receptor-dependent central functions of CCK in regulating motivation for alcohol consumption and related emotional behaviors [12, 77, 78]. More recently, Ballaz et al. thoroughly reviewed the hypothesis that endogenous CCK regulates alcohol intake, perhaps through interactions with DA, 5-HT, and endogenous opioid systems [3].

CCK neurocircuitry and neurotechnology

As described, early studies on CCK in addiction implicated sources of CCK in the ventral striatum, as well as NAcc-projecting VTA neurons that co-express CCK and DA, leading to multiple possibilities for CCK and DA interactions in modulating DA release and drug reward. Beyond these midbrain sites, recent investigations identified addiction-relevant functions of CCK throughout the basolateral amygdala (BLA) and extended amygdala (e.g., BNST). For example, multiple CCK neuronal subtypes exist in the BLA, including CCK + GABAergic interneurons that express type-1 cannabinoid receptors and facilitate behavioral extinction of fear memory, suggesting their potential role in drug reward memory and sensitivity to reinforcing properties of cannabinoids [62]. Though a full discussion on the role of CCK in stress and anxiety is beyond the scope of this review, the anxiogenic role of CCK2R signaling in the BLA is likely relevant to the stress-like withdrawal symptoms following cessation of chronic exposure to drugs of abuse [61].

In the extended amygdala, a dense band of CCK neurons is localized to the medial division of the BNST and sends long-range axonal projections to the lateral hypothalamus, medial amygdala, ventral pre-mammillary nucleus, and other brain areas regulating pursuit of natural rewards like food and sex [22]. Stimulation of CCK-BNST neurons by chemogenetic or optogenetic methods was positively reinforcing in male mice, and fiber photometry recordings revealed that CCK-BNST neurons were strongly activated by cues predicting rewarding stimuli, suggesting that BNST-derived CCK may promote drug-seeking behavior in addiction [24].

In addition to VTA-DA neurons, midbrain CCK is also expressed within multiple neuronal populations of the subaqueductal paramedian zone, including the dorsal raphe nucleus (DRN) [56]. Unlike archetypal 5HT cells of the DRN, single-cell RNA sequencing (sc-RNAseq) experiments indicated that CCK is expressed preferentially among DA-synthesizing neurons in the ventral periaqueductal gray that have been assigned to the DRN [35]. In other words, results from investigations of CCK-DA interactions throughout the classical mesolimbic pathway in addiction may also correspond to CCK-DA interactions through non-canonical pathways of DA DRN neurons that project to the VTA, hypothalamus, amygdala, and BNST [9].

In close proximity to the DRN, CCK is expressed in a separate midline group of peptidergic neurons called the centrally projecting Edinger-Westphal nucleus (EWcp) [57, 58, 71]. EWcp neurons are strongly activated by alcohol drinking and psychostimulants [23, 63, 64, 67], and expression of EWcp neuropeptides (including CCK at both the mRNA and protein levels) corresponds with the degree of voluntary alcohol intake among inbred mouse strains [20, 21, 25].

Altogether, the unique expression patterns of CCK in reward-promoting and alcohol-activated neurons of the BLA, BNST, DRN, and EWcp highlight the need for detailed investigations of the release and signaling mechanisms of CCK in these circuits to understand how this neuropeptide may be controlling discrete addiction-related behavioral processes (Fig. 1).

With this in mind, knowledge on the mechanisms regulating CCK release and signaling is limited. In mammals, release of neuropeptides have mostly been studied in the neurohypophysis, where large terminals contain high amounts of oxytocin or vasopressin large dense core vesicles (DCVs). Similarly, magnocellular neurons in the hypothalamus loaded with oxytocin DCVs have been a model system for understanding somatodendritic release of neuropeptides [73]. As vesicles containing other neuropeptides like CCK are typically smaller in size and number, it remains to be explored whether CCK shares similar release mechanisms. Spurred by the difficulty of measuring neuropeptide concentrations *in vivo*, recent breakthroughs in protein engineering have produced fluorescent receptor sensors that rely on dynamic green fluorescent protein (GFP) signals to report neuropeptide binding and receptor signaling with unprecedented specificity and temporal resolution [1, 69,85]. Using cell-specific viral approaches, targeting these neuropeptide sensors to defined neural pathways may profoundly alter the ability of scientists to detect and measure functional actions of peptides in behavioral animal models of addiction [27, 52, 66].

Summary and conclusions

Accumulating evidence over the past three decades suggest that the CCK system underlies a variety of behaviors related to substance use disorders. CCK actions across subcortical circuits are likely critical for emotional and reward-seeking aspects of addiction, with differential contributions of neuronal populations across the striatum, amygdala, and midbrain. The multifold nature of the endogenous CCK system and its role in addiction arise from gene polymorphism, peptide synthesis and post-translational processing, release modes, receptor subtypes with various signaling pathways, localization in terms of cell types and regions, and interaction with other neurotransmitter/neuromodulator systems. Given its wide distribution and high versatility, CCK is a promising candidate for unraveling the sex differences and hormonal influences on addictive behaviors. The challenges to detect CCK at physiological conditions have impeded the detailed investigation of CCK in addiction, but recent advances in neurotechnology offer genetic and optical methods complementary to traditional pharmacology. In conclusion, future studies with these sensitive and specific tools will provide novel insights of CCK and addition at a high level of granularity.

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Abbreviations:

BLA	Basolateral amygdala
BNST	Bed nucleus of stria terminalis
CCK	Cholecystokinin
DA	Dopamine
DCVs	Dense core vesicles
DRN	Dorsal raphe nucleus
EWcp	Centrally projecting Edinger-Westphal nucleus
E2	estradiol
GABA	Gamma-aminonbutyric acid
mRNA	Messenger ribonucleic acid
NAcc	Nucleus accumbens
5-HT	Serotonin
VTA	Ventral tegmental area

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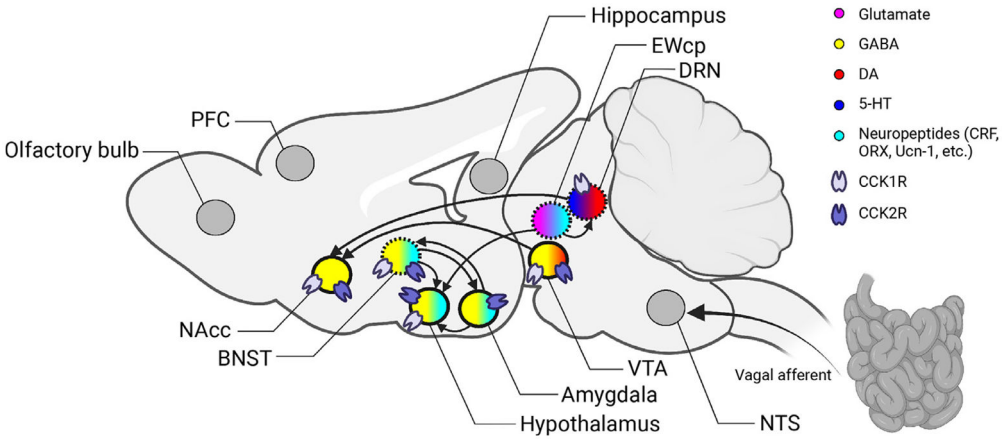

Fig. 1.

Depiction of CCK-containing neuronal cell bodies, as well as axonal projections and sites of CCK receptor signaling hypothesized to contribute to addiction to psychostimulants, opioids, and alcohol. Key subcortical regions are color-coded to show co-expressed neurotransmitters and neuromodulators. Other brain regions with known CCK function are labeled in gray. CRF, corticotropin-releasing factor; NTS, nucleus of the solitary tract; ORX, orexin; PFC, prefrontal cortex; Ucn1, urocortin-1. Created with BioRender.com.

Table 1

Summary of behavioral effects or molecular changes by altering CCK pathways in animal models of psychostimulant abuse. AMP, amphetamine; METH, methamphetamine; TH, tyrosine hydroxylase; DAT, dopamine transporter; CPu, caudate putamen; SN, substantia nigra; CPP, conditioned place preference.

Manipulation of CCK/CCKR	Brain Region	Molecular/Behavioral Effect	Animal Model	Refs.
CCK1/2R agonist (CCK-8)	n/a (i.c.v.)	• Attenuated METH-induced TH & DAT reduction in striatum/TH reduction in SN • Attenuated METH-induced hyper-locomotion, behavioral sensitization, and hyperthermia	♂C57BL/6 mice	[26]
CCK1R deficient	NAcc, CPu	• Higher baseline DA in CPu • Greater DA response in NAc to cocaine & AMP	OLETF rats	[19]
CCK1R antagonist (devazepide)	n/a (i.p.)	Blocked AMP-sensitized locomotion in high responders pre-exposed to AMP	♂Wistar rats	[16]
CCK1R antagonist (devazepide)	n/a (s.c.)	No effect on high responders		[29]
	NAcc	Inhibited cocaine-induced reinstatement of cocaine CPP	♂Sprague-Dawley rats	[47]
CCK1R antagonist (PD-140,548)	NAcc	Attenuated AMP-induced locomotor sensitization	♂Wistar rats	[79]
CCK2R agonist (CCK-5)	n/a (i.p.)	Increased cocaine intake	♂WP (water preferred) Wistar rats	[12]
CCK2R agonist (CCK-5)	NAcc	Increased AMP self-administration	♂Wistar rats	[8]
CCK2R antagonist (GV-150,013)	n/a (i.p.)	Reduced cocaine consumption	♂CD (cocaine drinking) Wistar rats	[13]
CCK2R antagonist (L-365,260)	n/a (s.c.)	Potentiated AMP-induced hyper-locomotion in low responders	♂Wistar rats	[30]
CCK2R antagonist (L-365,260)	NAcc, Amygdala	Inhibited stress-induced reinstatement of cocaine CPP	♂Sprague-Dawley rats	[47]
CCK2R antagonist (L-369,293)	CPu	Greater DA response in CPu to cocaine	♂Sprague-Dawley rats	[46]

Table 2

Summary of behavioral effects or molecular changes by altering CCK pathways in animal models of opioid abuse.

Manipulation of CCK/CCKR	Brain Region	Molecular/Behavioral Effect	Animal Model	Refs.
CCK1/2R agonist (CCK-8)	n/a (i.c.v.)	Blocked morphine CPP, attenuated CPP extinction, reduced locomotion	♂Wistar rats	[74]
CCK1/2R agonist (CCK-8)		• Inhibited anxiety-like behavior during morphine withdrawal • Effect can be inhibited by CCK1R antagonist (devazepide) & μ-opioid receptor antagonist (CTAP)		[75]
CCK1/2R agonist (CCK-8)		• Restored morphine-induced LTP reduction in dentate gyrus • Effect can be inhibited by CCK2R antagonist (L-365,260)		[81]
CCK1R antagonist(devazepide)	n/a (s.c.)	Blocked morphine CPP	♂Wistar rats	[30]
CCK2R agonist (BC264)	NAcc	Blocked morphine CPP	♂Lewis rats	[53]
CCK2R deletion	n/a	• Increased cAMP production induced by μ-opioid, δ-opioid, and D2 agonists • Hyperlocomotion at baseline and with morphine, more severe withdrawal signs	C57BL/6 J mice	[55]
CCK2R antagonist (L-365,260)	n/a (s.c.)	Potentiated low dose morphine CPP	♂Wistar rats	[31]
CCK2R antagonist (PD-134,308)	NAcc	Potentiated low dose morphine CPP	♂Fisher rats	[53]