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Review

Stress-Related Neuropeptides and Addictive Behaviors: Beyond the Usual Suspects

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Addictive disorders are chronic, relapsing conditions that cause extensive disease burden. Genetic factors partly account for susceptibility to addiction, but environmental factors such as stressful experiences and prolonged exposure of the brain to addictive drugs promote its development. Progression to addiction involves neuroadaptations within neurocircuitry that mediates stress responses and is influenced by several peptidergic neuromodulators. While corticotrophin releasing factor is the prototypic member of this class, recent work has identified several additional stress-related neuropeptides that play an important role in regulation of drug intake and relapse, including the urocortins, nociceptin, substance P, and neuropeptide S. Here, we review this emerging literature, discussing to what extent the properties of these neuromodulators are shared or distinct and considering their potential as drug targets.

Introduction

A major focus of drug addiction research has been on the neurocircuitry that mediates immediate positively reinforcing, or "rewarding," properties of drugs. However, it has become increasingly clear that progression to addiction also involves a shift to negatively reinforced drug seeking and taking, where drugs are pursued for their ability to alleviate aversive emotional states. Stress has emerged as an important trigger of relapse, and the neural systems that process stressful stimuli and coordinate psychological and physiological responses to them have become increasingly recognized as important factors that maintain the addicted state. Hypothalamic as well as extrahypothalamic corticotropin releasing factor (CRF, also known as CRH; see Table 1 for abbreviations) has received extensive attention as a mediator in this context and constitutes a prototype for a "stress-related neuropeptide" of critical importance for addictive processes (Heilig and Koob, 2007; Koob and Volkow, 2010; Koob and Zorrilla, 2010). Other neuropeptides with established roles in linking stress- and addiction-related behavior include dynorphin (Bruchas et al., 2010) and neuropeptide Y (NPY) (Heilig et al., 2010). More recently, however, additional neuropeptides including the urocortins (Ucns), neuropeptide S (NPS), nociceptin/orphanin FQ (N/OFQ), and neurokinins (NKs), have been implicated in processes that link stress responses with drug seeking, drug taking, and long-term neuroadaptations. In this Review, we focus on the involvement of stress-related neuropeptides in alcohol-related behaviors, also considering their contribution to stimulant and opioid-related processes when data are available.

Because the term "stress" has become so broadly and variably used in biology, some initial distinctions are necessary. First, the "stress" construct originates from material science, where it denotes an amount of external force, or load, that produces a corresponding measure of internal deformation, or "strain." In its expansion to biology, this distinction has been lost, and the term stress is applied both to the external forces that challenge the organism and the internal processes that result. Here, we will reserve the term "stress" and "stressors" for external demands placed on the organism. Second, "strain" in material science is a passive deformation. In contrast, biological organisms respond to external demands with a highly dynamic combination of physiological, emotional, cognitive, and behavioral responses that have evolved to be adaptive, although they may be more or less successful in a given instance. In the short term, re-establishing a pre-existing equilibrium, or homeostasis, is the classical example of a successful adaptive response, but other responses can clearly also be adaptive. For instance, eliminating the challenge altogether by moving away from it is an equally successful adaptation. In the following, we will denote this broader class of responses as "coping responses." Over time, maintaining stability by establishing a new setpoint, or "allostasis," may be viewed as an only partially successful adaptive response, which occurs in the face of prolonged stress exposure, at the cost of chronic wear and tear to the organism (McEwen and Gianaros, 2011). Henceforth, we will use the term "long-term neuroadaptations," or "neuroadaptations" for short, to denote the long-term changes that occur in the central nervous system in relation to this process.

Reward- and stress-related neural processes are frequently considered separately. However, a conceptualization informed by an evolutionary perspective helps highlight their intricate interrelationship. Approach and avoidance are broad classes of ancestral responses that guide an organism to emit behaviors in search of life-sustaining resources and to avoid harm, thus supporting survival (Alcaro and Panksepp, 2011; Korte et al., 2005). Accordingly, approach and avoidance systems are highly

Table 1. Abbreviations

5-HT	Serotonin
ACTH	Adrenocorticotropic hormone
AMG	Amygdala
BG	Basal ganglia
BLA	Basolateral nucleus of the amygdala
BNST	Bed nucleus of the stria terminalis
CeA	Central nucleus of the amygdala
CPP	Conditioned place preference
CRF	Corticotropin releasing factor
CRH	Corticotropin releasing hormone
CRF_1R	CRF type-1 receptor
CRF_2R	CRF type-2 receptor
CRFBP	CRF binding protein
DA	Dopamine
DR	Dorsal raphe nucleus
EPM	Elevated plus maze
EWcp	Centrally projecting Edinger-Westphal nucleus
HPA axis	Hypothalamic-pituitary-adrenal axis
HYP	Hypothalamus
ICV	Intracerebroventricular
LC	Locus coeruleus
LH	Lateral hypothalamus
LS	Lateral septum
ICV	Intracerebroventricular
LPB	Lateral parabrachial nucleus
MSN	Medium spiny neuron
NAC	Nucleus accumbens
NE	Norepinephrine
NK	Neurokinin
NK_1R	Neurokinin 1 receptor
NK_2R	Neurokinin 2 receptor
NK_3R	Neurokinin 3 receptor
NKA	Neurokinin A
NKB	Neurokinin B
NOPR	Nociceptin/orphanin FQ receptor (also ORL1: opioid receptor-like 1 receptor)
N/OFQ	Nociceptin/orphanin FQ
NPS	Neuropeptide S
NPSR	Neuropeptide S receptor
NPY	Neuropeptide Y
SN	Substantia nigra
SP	Substance P
STR	Striatum
Ucn	Urocortin
Ucn1	Urocortin 1
Ucn2	Urocortin 2
Ucn3	Urocortin 3
VTA	Ventral tegmental area

conserved. Their neuroanatomical substrates are phylogenetically old, such as the basal ganglia (BG), the amygdaloid complex, the hypothalamus (HYP), and other conserved structures of the brain. In addition, as nonhuman primates and humans left their ecological niches and became able to adapt to a broader range of environmental conditions, the neocortex evolved an ability for more flexibly shaping approach and withdrawal responses, suggesting that unique features may distinguish these species (Noonan et al., 2012).

A fundamental aspect of coping in a diverse environment is to switch between motivational processes that drive appetitive approach responses and those that promote avoidance (Alcaro and Panksepp, 2011; Korte et al., 2005). Stress mechanisms have a critical role in shaping this behavioral flexibility. CRF is a prototypical neuropeptide that predominantly promotes withdrawal and attenuates appetitive behaviors, while NPY has the opposite profile. The interrelationship of these two prototypical neuropeptides can be conceptualized in a relatively straightforward manner as mediators of these opponent processes, and key elements of the neurocircuitry mediating their interactions, such as the amygdala (AMG) complex, have been recognized for some time (Heilig et al., 1994). The Ucns, NKs, N/OFQ, and NPS have activity profiles that in part fall into these prototypical categories but also differ from them in being more complex. Here, we will review key findings on each of the individual systems, discuss their similarities and differences, attempt to integrate their interrelationship and the anatomical structures through which they may interact, and identify knowledge gaps that need to be filled.

CRF-Related Ucn Peptides

Basic Features of Ucn Systems

The first member of the CRF/Ucn family to be isolated, CRF, was originally discovered for its crucial role in activation of the hypothalamic-pituitary-adrenal (HPA) axis (Vale et al., 1981). Subsequently, CRF was shown to also mediate a broad range of coordinated physiological and behavioral stress responses, as well as neuroadaptations that contribute to the development of addiction (Heilig and Koob, 2007; Koob and Zorrilla, 2010; Shalev et al., 2010). With the discovery of Ucn:s (Ucn1, Ucn2, and Ucn3), it has become clear that the complexity of the CRF/Ucn system is greater than initially appreciated (Lewis et al., 2001; Lovenberg et al., 1995; Potter et al., 1991; Reyes et al., 2001; Vaughan et al., 1995). While the Ucn:s share 20%–45% sequence homology with CRF, physiological functions of CRF/Ucn family peptides are not highly conserved. For example, Ucn2 and Ucn3 do not directly influence stress reactivity but instead alter social behaviors in mice, suggesting that mammals have adapted these peptides for regulation of social interactions (Breu et al., 2012; Deussing et al., 2010). Figure 1 presents a schematic of the contribution of the Ucn system to stress- and addiction-related behaviors.

CRF type-1 and CRF type-2 receptors (CRF_1R and CRF_2R) are both members of the class B/secretin family of heptahelical receptors and are encoded by *Crhr1* and *Crhr2* genes, respectively. The *Crhr2* gene gives rise to at least two alternatively spliced isoforms: $CRF_{2(a)}$, expressed in neurons, and $CRF_{2(b)}$, expressed in peripheral tissues and nonneuronal brain structures

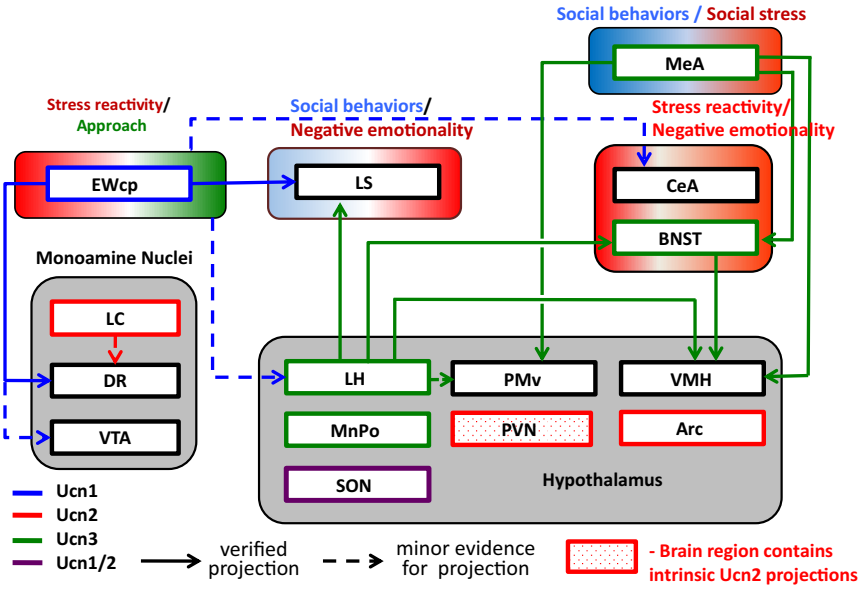

Figure 1. Ucn Circuitry Potentially Impacting Addiction-Related Behaviors

EWcp is the main site of Ucn1 production in brain. Ucn1 projections (blue) from this structure to the LS and DR, two structures that mediate behavioral stress responses, are well established. Ucn1-immunoreactive fibers are, however, widely distributed, and the number of Ucn1-innervated brain regions is probably much greater than depicted. Projections from Ucn2 cell bodies (red) largely await characterization, while much of the forebrain Ucn3 circuitry has been mapped and is indicated (green). Ucn1 activates both CRF_1R and CRF_2R, while Ucn2 and Ucn3 are CRF_2R selective (see main text). CRF_1R are widely distributed in the brain and are not shown; areas indicated in the figure as targets of Ucn peptides all contain CRF_2 receptors to various degrees. Endogenous Ucn1 pathways originating from the EWcp mediate positively reinforcing effects of alcohol, although exogenous Ucn1 administration inhibits alcohol intake. The latter effect presumably reflects actions on targets that are innervated by endogenous Ucn2 or Ucn3 pathways, since exogenous administration of the latter two peptides inhibits alcohol intake. Endogenous Ucn2 or Ucn3 positively regulates the acute locomotor response to methamphetamine, a response that involves AMG neurons. Brain stem and peripheral targets of Ucns are not shown. (MnPO, median preoptic area; SON, supraoptic hypothalamic nucleus; PMv, ventral premammilary hypothalamus; PVN, paraventricular hypothalamic nucleus; VMH, ventromedial hypothalamic nucleus; Arc, arcuate nucleus of hypothalamus).

(Bale and Vale, 2004). $CRF_{2(a)}$ and CRF_1 receptors share approximately 70% amino acid sequence homology, with a particularly high degree of conservation in regions thought to be the primary site of G protein coupling and signal transduction. Functional specificity of the CRF receptors appears to arise from their distinct cellular expression patterns, anatomical distributions, or both.

CRF is largely a CRF_1R agonist and displays 18-fold greater affinity for CRF_1R than CRF_2R (Vaughan et al., 1995). In contrast, Ucn:s are high-affinity agonists for CRF_2R, with varying degrees of affinity for CRF_1R. Ucn1 binds both receptor subtypes with high affinity, and Ucn1-positive fibers innervate regions expressing both receptors, while Ucn2 and Ucn3 are highly CRF_2R selective (Bittencourt et al., 1999; Fekete and Zorrilla, 2007). The Ucn:s also have varying affinity for the CRF binding protein (CRFBP): Ucn1 binds to the CRFBP, while Ucn3 does not, and the affinity of Ucn2 for CRFBP is species dependent (Fekete and Zorrilla, 2007). Though early findings showed that CRFBP inhibits CRF/CRF_1R signaling (Potter et al., 1991), more recent data suggest that interactions with CRFBP may be required for some actions of CRF at the CRF_2R (Ungless et al., 2003; Wang et al., 2007).

CRF_2Rs have a more restricted distribution than the CRF_1 subtype and are primarily localized to structures involved in behavioral stress responses, including: the dorsal raphe (DR) nucleus, lateral septum (LS), bed nucleus of the stria terminalis BNST, AMG, and HYP, (Cavalcante et al., 2006; Chalmers et al., 1995; Chen et al., 2011; Kuperman et al., 2010; Li et al., 2002; Van Pett et al., 2000). Some CRF_2 receptor-expressing regions receive innervation from multiple sites containing different CRF/Ucn ligands, and studies using pharmacological tools may therefore be insufficient to identify the functional role of the respective endogenous input.

Ucn:s and Stress Responses

Effects of Ucn:s on stress responses are more restricted but also more complex than those of CRF. In contrast to CRF, Ucn:s do not play a direct role in HPA axis responses (Kageyama et al., 2003; Nemoto et al., 2009). Ucn/CRF_2R activation has repeatedly been shown to result in reduction of anxiety-like behavior (anxiolysis) and recovery from stress (Coste et al., 2000; Tanaka and Telegdy, 2008; Todorovic et al., 2007; Valdez et al., 2003), i.e., effects opposite those mediated by CRF through actions at CRF_1Rs. However, CRF_2R signaling can also drive stress-induced increases in anxiety (Henry et al., 2006), aversion (Land et al., 2008), and alcohol consumption (Pastor et al., 2011), while social defeat stress potently activates CRF_2R-expressing neurons of the medial AMG (Fekete et al., 2009). Ucn:s also play a role in long-term stress adaptation (Neufeld-Cohen et al., 2010a, 2010b). It is clear from the complexity of functional consequences that Ucn/CRF_2R signaling does not serve simply as an ''antialarm'' system opposing CRF actions.

Ucn:s in Regulation of Alcohol Consumption

Converging lines of evidence indicate that endogenous Ucn1 promotes alcohol consumption (Bachtell et al., 2003; Giardino et al., 2011a; Ryabinin et al., 2012; Ryabinin and Weitemier, 2006). In rodents, Ucn1-containing neurons within the centrally projecting Edinger-Westphal nucleus (EWcp) are particularly sensitive to voluntary alcohol consumption (Anacker et al., 2011; Bachtell et al., 2003; Kaur and Ryabinin, 2010; Ryabinin et al., 2003; Weitemier et al., 2001). The neuropeptide-containing neurons of the EWcp send Ucn1-positive axons to brain regions that include the LS and DR, structures involved in behavioral stress responses (Bachtell et al., 2004; Bittencourt et al., 1999; Kozicz et al., 2011). Higher levels of EWcp-Ucn1 protein were associated with higher alcohol consumption and alcohol-induced reward in rodent strains that vary in alcohol-related

behaviors (Bachtell et al., 2003; Fonareva et al., 2009; Kiianmaa et al., 2003; Ryabinin and Weitemier, 2006; Turek et al., 2005). A recent comparison of alcohol-preferring C57BL/6J mice and alcohol-avoiding DBA/2J mice showed that in these lines, differences in Ucn1 peptide levels were due to increased EWcp-Ucn1 mRNA levels (Giardino et al., 2012a).

A functional role for EWcp-Ucn1 neurons in alcohol consumption is supported by findings that electrolytic lesions of the mouse EWcp decreased alcohol preference in a Ucn1-dependent manner (Giardino et al., 2011a). This issue has, however, been complicated by findings in which exogenous administration of Ucn:s decreased alcohol intake in nondependent mice (Lowery et al., 2010; Ryabinin et al., 2008; Sharpe and Phillips, 2009). It was recently shown that genetic deletion of Ucn1 blunts alcohol preference and alcohol-induced reward but does not influence alcohol-induced aversion (Giardino et al., 2011a). In nondependent animals, the net effect of endogenous Ucn1 activity is to promote alcohol consumption, but this seems to be mediated through appetitive rather than aversive, stress-related mechanisms.

As alcohol dependence evolves, alcohol consumption escalates. This is thought to be associated with a shift from alcohol consumption for rewarding, positively reinforcing properties, to intake driven by stress-dampening, negatively reinforcing alcohol effects. Recent data show that Ucn1 contributes to the progressive escalation of alcohol preference seen during long-term intermittent access (Giardino and Ryabinin, 2012, Alcohol. Clin. Exp. Res., abstract), suggesting that, similar to the CRF/CRF_1R system (Heilig and Koob, 2007), the Ucn/CRF_2R system may also undergo neuroadaptations as addictive processes evolve. Interestingly, intra-amygdalar injections of the highly selective CRF_2 ligand Ucn3 increased alcohol self-administration in nondependent rats but suppressed it in rats made chronically dependent on alcohol (Funk and Koob, 2007). An involvement of the Ucn/CRF_2 system in dependence-related neuroadaptations is further supported by the observation that the expression of CRF_2Rs in the AMG was downregulated after a history of alcohol dependence (Sommer et al., 2008).

In summary, motivational mechanisms that mediate the role of Ucn peptides and CRF_2R activation on alcohol consumption are presently less well understood than those of CRF_1Rs and may involve both stress- and reward-related mechanisms. The relative contribution of individual Ucn:s in different brain regions, and in different stages of addiction-related processes, also remains to be established. More work is needed to assess the potential of CRF_2R ligands as alcoholism pharmacotherapies, determine in what stage of the disease process they may be most useful, and define their optimal pharmacological profile. Due to the bidirectional effects of CRF_2R agonists on alcohol consumption, region-specific manipulations of endogenous Ucn:s will be required to dissect their relative involvement in motivation to seek and consume alcohol and in the transition to alcohol dependence.

Ucn:s and Other Addictive Drugs

Chronic cocaine has been shown to switch CRF_2R modulation of glutamatergic transmission from inhibitory to excitatory in the LS (Liu et al., 2005), but the consequences of this plasticity for stress responses and drug seeking remain to be determined. The LS has long been held to play a role in emotional processes and stress responses, and neurons within the LS promote active stress-coping behavior and inhibit HPA axis responses to stress (Singewald et al., 2011). CRF receptors within the LS are predominantly of the CRF_2 type, and blockade of these receptors has been shown to result in a specific reduction in stress-induced behavior, while their stimulation promotes anorexia and anxiety-like behavior (Bakshi et al., 2007). Modulation of LS function by CRF_2 receptors may, however, also impact drug seeking driven by rewarding, appetitive processes, because a pathway that originates in the LS drives hypothalamic hypocretin/orexin neurons and is necessary for cocaine conditioned place preference (CPP) (Sartor and Aston-Jones, 2012).

CRF_2R as well as CRF_1R are present within the DR, a structure that modulates behavioral stress responses through serotonergic projections to widespread target areas in the forebrain (Waselus et al., 2011). CRF_1Rs and CRF_2Rs have opposing effects on serotonin (5-HT) release in projection areas of serotonergic DR neurons (Lukkes et al., 2008). Withdrawal from chronic stimulants is associated with increased sensitivity to stress and negative emotional states both in humans and animals, and these states are thought to contribute to increased relapse vulnerability. The CRF_2R was found to be elevated in the DR after chronic amphetamine treatment (Pringle et al., 2008), and intra-DR CRF_2R blockade dampened the enhanced anxiety-like behavior observed during amphetamine withdrawal (Vuong et al., 2010). This suggests that CRF_2R antagonists may have a potential to prevent motivational consequences of negative emotional states and CRF_2R upregulation resulting from stimulant use.

Similar to the findings with alcohol, Ucn:s may also influence stimulant drug seeking and consumption through actions on systems that mediate approach behavior rather than avoidance. It is well established that mesolimbic dopamine (DA) neurons originating in the ventral tegmental area (VTA) are critical for exploration and approach behaviors (Koob and Volkow, 2010). Electrophysiological experiments on VTA slice preparations found that bath application of CRF potentiates NMDA receptor (NMDAR)-mediated excitatory postsynaptic currents in DA neurons, an effect that was blocked by CRF_2R but not CRF_1R antagonists (Ungless et al., 2003). This finding was surprising, because mRNA for CRF_2R had not been detected in the VTA by in situ hybridization (Van Pett et al., 2000). Subsequent single-cell RT-PCR data suggested that CRF_2R transcript is expressed in VTA DA neurons, although perhaps at levels too low to be detected by in situ (Ungless et al., 2003). The presence of CRF_2R in the DA neurons of the VTA has remained controversial (Wise and Morales, 2010), but it has been shown that CRF_2R is required for potentiation of NMDAR transmission and Ca^{2+} release in these cells (Riegel and Williams, 2008; Ungless et al., 2003).

Although typically associated with approach behaviors, the VTA is also engaged in stress-induced reinstatement of drug seeking. It has been reported that intra-VTA CRF_2R blockade dampens stress-induced reinstatement of cocaine seeking (Wang et al., 2007), but another study failed to replicate these

results (Blacktop et al., 2011). In this report, both CRF and foot-shock stress-induced reinstatement of cocaine seeking were blocked by VTA injections of two different selective CRF_1R antagonists but not two CRF_2R antagonists. Furthermore, the CRF_1R-selective agonist cortagine, but not the CRF_2R-selective agonist Ucn2, replicated the effects of CRF to reinstate cocaine seeking. These data are in agreement with previous findings that systemic or intracerebroventricular (ICV) injections of CRF_1R, but not CRF_2R antagonists, block stress-induced reinstatement (Lu et al., 2003). Taken together, a clear role of CRF_2R and Ucn:s in cocaine-seeking behavior is yet to be established.

Finally, recent studies identified a role for CRF_2R in the acute locomotor response to methamphetamine, which was associated with CRF_2R-dependent neural activation within the central amygdala (CeA) and basolateral amygdala (BLA) (Giardino et al., 2011b). In contrast, the locomotor effects of cocaine were sensitive to deletion of CRF_1R, but not CRF_2R (Giardino et al., 2012b). Although EWcp-Ucn1 neurons are transcriptionally activated in response to both amphetamines and cocaine (Spangler et al., 2009), the acute response to methamphetamine is not dampened by genetic deletion of Ucn1, indicating that Ucn2 or Ucn3 is involved in this behavior. Thus, CRF_2Rs may be differentially involved in locomotor effects of different stimulants.

Evaluating the Potential of Ucn/*CRF_2R* Systems as Therapeutic Target

Blocking CRF activity via CRF_1R antagonism remains an attractive principle for addiction pharmacotherapy, and initial clinical development targeting this mechanism is now underway (see e.g., http://www.clinicaltrials.gov, NCT01227980). The complex actions of Ucn:s hold the promise of offering additional opportunities for developing addiction treatments. Because their relative preference for CRF_2R relative to CRF_1Rs differs, understanding the role of individual Ucn:s will provide important clues to the optimal properties of therapeutics that could be developed to target Ucn/CRF_2R systems. The lack of selective nonpeptide ligands for the CRF_2R is a limitation in this regard, and developing selective molecules to target this receptor is an important research priority.

N/OFQ and Its Receptor (NOPR)

Basic Features of the N/OFQ-NOPR System

N/OFQ, a 17 amino acid neuropeptide that is structurally related to the opioid peptide dynorphin A, originates from proorphanin, a larger peptide encoded by the preproorphanin gene (Meunier et al., 1995; Reinscheid et al., 1995). N/OFQ and its receptor, NOPR, are widely expressed in the brain, where they control the release of other neurotransmitters through presynaptic actions (Darland et al., 1998; Neal et al., 1999).

Despite its structural homology with opioid peptides, N/OFQ does not bind to the opioid receptors and, conversely, opioid peptides do not activate the NOPR (Reinscheid et al., 1996). Additionally, while opioid-like N/OFQ elicits pronociceptive effects after intracranial administration, giving rise to the name nociceptin (Meunier et al., 1995), and acts in the brain to produce functional antiopioid effects, it blocks opioid-induced supraspinal analgesia (Mogil et al., 1996), morphine-induced CPP (Ciccocioppo et al., 2000; Murphy et al., 1999), and morphine-induced increases in extracellular DA levels in the nucleus accumbens (NAC) (Di Giannuario and Pieretti, 2000).

N/OFQ/NOPR System and Stress Responses

Activation of NOPR produces anxiolytic-like effects (Gavioli and Calo', 2006; Varty et al., 2005) that appear to be particularly robust under stressful conditions, such as during alcohol withdrawal (Economidou et al., 2011). This may depend upon the ability of N/OFQ to act as a functional antagonist for extrahypothalamic actions of CRF and CRF_1R activation. For instance, it has been shown that N/OFQ blocks the anorectic and the anxiogenic-like effects of CRF, with the BNST being the site of the interaction between the two systems (Ciccocioppo et al., 2003; Rodi et al., 2008). In addition, N/OFQ opposes the ability of CRF to facilitate GABAergic transmission in the CeA, an effect that is more pronounced in slice preparations from rats undergoing alcohol withdrawal, a state known to be associated with enhanced stress reactivity and overactive CRF neurotransmission (Cruz et al., 2012). These data provide converging evidence supporting the possibility that NOPR activation may result in particularly beneficial antistress and anxiolytic-like effects when the CRF system is activated. This view is supported by gene expression data showing that exposure to stressful conditions, such as alcohol withdrawal or intracranial CRF administration, leads to upregulated NOPR expression in the BNST, which may explain in part the enhanced efficacy of N/OFQ to produce antistress effects under these conditions (Martin-Fardon et al., 2010; Rodi et al., 2008).

The N/OFQ-NOPR System and Alcohol-Related Behaviors

Several studies have demonstrated that activation of the NOPR blunts the reinforcing and motivational effects of alcohol across a range of behavioral measures, including alcohol intake (Ciccocioppo et al., 1999), CPP (Kuzmin et al., 2003), and relapse to alcohol seeking triggered by alcohol-associated cues (Ciccocioppo et al., 2004) or stress (Martin-Fardon et al., 2000). The latter result is particularly noteworthy, because relapse-like behavior triggered by stress or cues are otherwise to a large degree pharmacologically dissociable (Shalev et al., 2002). Neurocircuitry mediating aversive emotional states is implied in stress-induced relapse by the ability of CRF_1R antagonists to block this behavior. In contrast, appetitive mechanisms are implied in cue-induced relapse to alcohol seeking, since it is blocked by the mu opioid receptor-preferring antagonist naltrexone, which also blocks ongoing alcohol self-administration in nondependent rats (Lê et al., 1999, 2000; Liu and Weiss, 2002). The ability of N/OFQ to block both stress- and cue-induced relapse therefore raises two distinct possibilities. One is that N/OFQ simply acts at multiple sites in the brain to modulate both aversive and appetitive motivations (Figure 4). Alternatively, it has been suggested that neurocircuitry-mediating relapse triggered by stress- and drug-associated cues converges on a common final output pathway (Kalivas and Volkow, 2005), and N/OFQ may act beyond that point of convergence.

Genetically selected alcohol-preferring rats are particularly sensitive to suppression of alcohol drinking and relapse by NOPR agonists (Ciccocioppo et al., 1999, 2004; Economidou et al., 2008). These rats exhibit high innate sensitivity to stress

and high measures of both anxiety- and depression-like behaviors that are ameliorated by alcohol consumption (Ciccocioppo et al., 2006; Ciccocioppo and Hyytia, 2006). Hence, the effects of N/OFQ are in part likely due to its ability to alleviate a negative emotional state that otherwise provides an incentive for negatively reinforced alcohol consumption. Notably, these rats appear to have an innate upregulation of the N/OFQ-NOPR system in several brain regions, and there appears to be a partial uncoupling of the NOPR from G protein-mediated signal transduction in the CeA that may lead to a regionally selective functional deficit of the N/OFQ system, which could contribute to high levels of alcohol drinking and anxiety-like behavior (Economidou et al., 2008). This hypothesis is corroborated by data showing that alcohol self-administration is reduced by site-specific injections of N/OFQ into the CeA (Economidou et al., 2008).

In a recent study, it was also shown that intracranial N/OFQ administration abolished somatic withdrawal signs during acute withdrawal and significantly attenuated anxiety-like behavior during protracted abstinence (Economidou et al., 2011). These data suggest that, in addition to their potential as medications for excessive alcohol consumption and relapse, agonists for NOPRs may also have the utility to treat alcohol withdrawal. Wistar rats tested for alcohol self-administration 1 week after withdrawal from chronic dependence were more sensitive both to the alcohol intake-reducing and to the anxiolytic-like actions of N/OFQ than nondependent control rats (Aujla et al., 2012; Economidou et al., 2011; Martin-Fardon et al., 2010). However, 3 weeks into abstinence, ICV N/OFQ administration resulted in anxiogenic-like effects in rats with a history of alcohol dependence, while it continued to exert anxiolytic-like actions in controls.

N/OFQ-NOPR System and Other Addictive Drugs

Less is known about potential antiaddictive properties of N/OFQ in relation to other drugs of abuse. It has been shown that N/OFQ prevents the expression of CPP for cocaine, methamphetamine, and morphine (Ciccocioppo et al., 2000; Kotlińska et al., 2002; Murphy et al., 1999; Zhao et al., 2003). Accordingly, microdialysis experiments have shown that intracranial N/OFQ injections prevent cocaine- and morphine-induced increases in extracellular DA within the NAC (Di Giannuario and Pieretti, 2000; Lutfy et al., 2001). Indirect evidence supporting the ability of N/OFQ to attenuate the rewarding effect of drugs of abuse also comes from studies on NOPR null mutant mice, which had increased sensitivity to the rewarding effects of cocaine, morphine, and nicotine (Marquez et al., 2008; Rutten et al., 2011; Sakoori and Murphy, 2009). For a better assessment of their potential antiaddictive properties in relation to these drugs, however, NOPR agonists need to be examined using self-administration and reinstatement experiments. One study has examined the effects of N/OFQ on stress-induced reinstatement of cocaine seeking under operant conditions, and the results were negative (Martin-Fardon et al., 2000).

Evaluating the Potential of N/OFQ-NOPR System as Therapeutic Target

The results reviewed above suggest that selective NOPR agonists may represent a promising strategy to treat addiction, particularly in alcoholism. Nonpeptide, orally available, and brain-penetrant NOPR agonists have been developed and seem to have acceptable safety and tolerability. Some of these may soon become ready for clinical evaluation.

SP and the NK Receptors

Basic Features of the SP/NK_1R System

SP is an 11 amino acid member of the tachykinin family, which also includes neurokinin A (NKA) and neurokinin B (NKB) (Pennefather et al., 2004). Three receptor subtypes exist for these neuropeptides, with SP preferentially binding to the neurokinin 1 receptor (NK_1R), while the neurokinin 2 receptor (NK_2R) is preferentially activated by NKA and neurokinin 3 receptor (NK_3R) by NKB. NK_1Rs are located in a range of brain regions involved in both appetitive and aversive behaviors (Figure 2).

The NK_1R was the first neuropeptide receptor for which a potent, highly selective nonpeptide antagonist was developed (Snider et al., 1991). Subsequent drug development efforts targeting this receptor were in part complicated by the fact that it displays considerable divergence between species, and many compounds that have high affinity for the human NK_1R do not effectively bind the rat NK_1R (Jensen et al., 1994; Leffler et al., 2009). NK_1R antagonists have been explored for the treatment of inflammatory conditions, depression, and chemotherapy-induced nausea (for review, see e.g., Quartara et al., 2009). With one exception, the treatment of chemotherapy-induced nausea, efforts targeting NK_1R have not resulted in therapeutics approved for clinical use. Although previous attempts to develop NK_1R antagonists for depression were unsuccessful, recent studies have provided renewed support for their antidepressant potential but indicated that near-complete central receptor occupancy might be required to achieve this effect (Ratti et al., 2011; Zamuner et al., 2012).

SP/NK_1R and Stress Responses

The SP/NK_1R system regulates stress- and anxiety-related behaviors (reviewed in Ebner and Singewald, 2006). NK_1R antagonists have anxiolytic-like properties, even under basal, nonstressed conditions (Ebner et al., 2008a; Santarelli et al., 2001). Effects of NK_1R activation by SP on stress-related behaviors are ultimately likely to be mediated through postsynaptic actions and modulation of other transmitter systems, but NK_1R also has a bidirectional effect on SP release itself (Singewald et al., 2008). NK_1R activation suppresses SP release within the AMG at baseline but stimulates it during acute stress exposure. This shift is hypothesized to result from volume transmission during stress exposure, resulting in activation of extrasynaptic NK_1Rs (or other NK receptor subtypes with lower affinity for SP) versus synaptically restricted transmission at rest. Interestingly, it has been demonstrated that NK_1Rs in the striatum (STR) are mostly extrasynaptic (Pickel et al., 2000), but this has not yet been confirmed in the AMG.

In agreement with its role in stress responses, the SP/NK_1R system also contributes to the regulation of the HPA axis. SP administration can enhance stress-induced corticosterone release (Mello et al., 2007) and expression of CRF_1R (Hamke et al., 2006). Furthermore, anxiety-like responses and mild stress-induced elevations in corticosterone are blunted in mice with genetic deletion of the NK_1R (Santarelli et al., 2001). The paraventricular nucleus of the hypothalamus, a region that drives

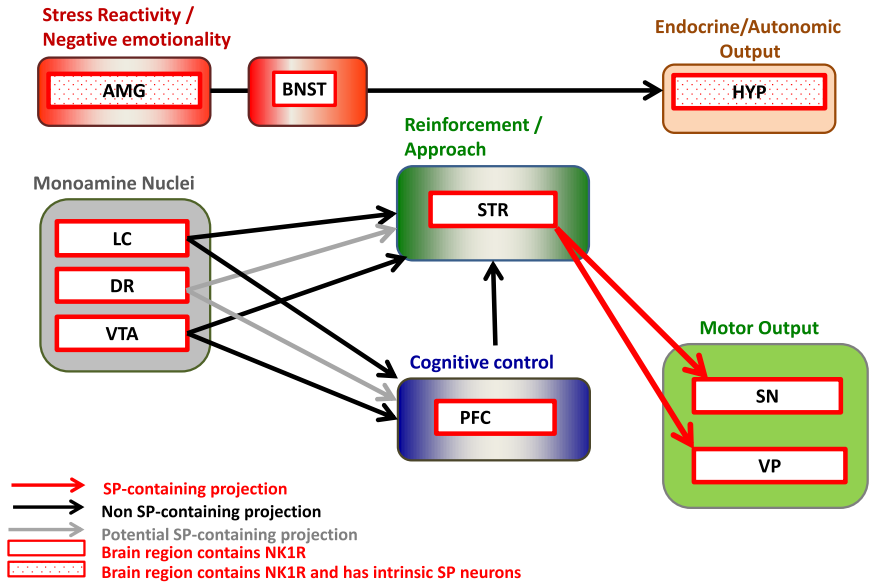

Figure 2. SP/NK_1R Circuitry Potentially Impacting Addiction-Related Behaviors

Brain regions that receive SP projections and contain NK_1Rs to varying degrees are shown. SP and NK_1Rs have been shown to regulate the activity of brainstem and midbrain monoamine nuclei (norepinephrine [NE] neurons of LC, DA neurons of VTA, and 5-HT neurons of DR), which have widespread projections to forebrain regions; some of these, relevant for addiction-related behaviors, are included in the current schematic. Some 5-HT neurons of the DR coexpress SP, but target regions of this subset are not established; the projections that contain 5-HT potentially colocalized with SP are shown in gray. The AMG and HYP, which regulate behavioral, autonomic, and endocrine stress responses, contain intrinsic SP circuits that modulate their output. The pathway from the prefrontal cortex (PFC) to the NAC (which is part of the STR core subregion) to the VP is part of a proposed "final common pathway" for reinstatement of drug seeking (see main text). MSNs of the NAC that project to the VP and SN contain SP and activate NK_1R and/or NK_3R in these regions. These SP-containing GABAergic projections are shown in red. Not shown is the habenula, an NK_1R-containing structure recently postulated to mediate important antireward processes; a role of SP and NK_1R in these has not yet been evaluated.

HPA axis activity and stress-induced autonomic activation, receives input from SP-positive fibers (Kawano and Masuko, 1992; Womack and Barrett-Jolley, 2007; Womack et al., 2007), and NK_1R antagonists can suppress stress-induced c-*fos* activation in this region (Ebner et al., 2008a). There has been some suggestion that NKR antagonist administration can increase adrenocorticotropic hormone (ACTH) and CRF expression and release (Jessop et al., 2000), while SP can suppress ACTH release (Jones et al., 1978). However, the majority of the findings outlined above suggest a facilitory role of NK_1R stimulation on HPA axis activity during stress. In humans, SP-mediated stimulation of the HPA axis appears to dominate, because administration of an NK_1R antagonist over the course of several weeks did not influence basal cortisol levels but did block stress-induced release of both ACTH and cortisol (George et al., 2008).

The NK_1R also modulates monoaminergic transmission after stress exposure. During forced-swim stress, NK_1R antagonism promotes active coping behavior and prevents the suppression of 5-HT release in the LS that is normally seen under these conditions (Ebner et al., 2008b). SP is released in response to stress, and it has been shown that NK_1R activation suppresses DR activity and 5-HT release (Guiard et al., 2007; Valentino et al., 2003) and that genetic or pharmacological inhibition of the NK_1R can increase serotonergic activity (Conley et al., 2002; Gobbi et al., 2007; Santarelli et al., 2001). In addition, NK_1Rs are also present on the noradrenergic cell bodies of the locus coeruleus (LC) (Chen et al., 2000; Ma and Bleasdale, 2002) and dynamically regulate the activity of this nucleus. The ability of NK_1Rs to modulate noradrenergic transmission is especially intriguing, as this system is involved in stress-induced reinstatement of drug seeking and escalated self-administration of multiple classes of drugs.

SP and NK_1R at the Intersection of Stress and Reward

In addition to the role in stress responses reviewed above, effects of NK_1R activation on catecholamine signaling in the mesolimbic, mesocortical, and nigrostriatal pathways also suggest a role in appetitive behaviors, including those related to drug seeking and taking. The catecholamine DA is classically associated with rewarding properties of addictive drugs and interacts with SP in pathways that drive drug seeking. For example, SP is colocalized with the D_1 receptor in a subpopulation of medium spiny neurons (MSNs) of the ventral STR (Le Moine and Bloch, 1995). The majority of these neurons feed back onto the substantia nigra (SN), a region that contains dopaminergic cell bodies and expresses NK_1Rs (Futami et al., 1998; Le Moine and Bloch, 1995; Whitty et al., 1995). Infusion of SP or SP analogs into the SN or VTA stimulates the firing rate of these neurons and subsequent DA release in their terminal fields (Barnes et al., 1990; West and Michael, 1991), increases locomotor activity (Barnes et al., 1990; Eison et al., 1982; Elliott et al., 1992; Kelley et al., 1979; Placenza et al., 2004), and induces CPP (Boix et al., 1995; Nikolaus et al., 1999). The relative contribution of NK receptor subtypes to the effects of SP in the VTA and SN remains unclear, as the NK3R may also play a role. Another subset of SPergic MSNs of the ventral STR project to the ventral pallidum (VP) (Lu et al., 1998), a brain region involved in drug seeking as part of a final common pathway for relapse (see Kalivas and Volkow, 2005). The NK_1R is also located throughout the STR, where it is found on dendrites of cholinergic interneurons as well as terminals projecting into this region (Commons and Serock, 2009; Murtra et al., 2000; Pickel et al., 2000).

Tachykinin systems have been highly conserved throughout evolution, and SP is found in the BG of all vertebrates (Holmgren and Jensen, 2001; Medina and Reiner, 1995; Smeets et al.,

2000). The activity of SP in these regions suggests that it contributes to the execution of motivated behaviors. SP and its NK_1R are therefore positioned at the intersection of appetitive and aversive behaviors and provide a substrate by which these behaviors can interact. In considering specific effects of manipulating this system on drug seeking and taking, there is therefore a need to carefully consider whether effects are produced through actions that impact reward- or stress-related circuitry or both.

SP/NK_1R System in Responses to Opioids and Psychostimulants

Manipulations of the SP/NK_1R system have been shown to influence several addiction-related behaviors. For example, NK_1R knockout mice do not display morphine-CPP and self-administer morphine at lower rates. Morphine-induced locomotor activation and psychomotor sensitization are also blunted in these mice (Murtra et al., 2000; Ripley et al., 2002). Lesions of NK1R-containing neurons in the AMG, but not NAC, suppressed morphine-induced CPP, a finding suggesting that NK1Rs in the AMG contribute to rewarding properties of morphine (Gadd et al., 2003). Reduced opioid reward after NK_1R blockade was recently also supported by observations that this treatment attenuates the ability of morphine to lower intracranial self-stimulation thresholds (Robinson et al., 2012). Coadministration of SP and morphine prevents the internalization and acute desensitization of the mu opioid receptor typically induced by morphine, which may account for the involvement of the NK_1R in opioid reward (Yu et al., 2009).

These data collectively support a role of NK_1R activation in rewarding properties of opioids and suggest the possibility that NK_1R antagonists may be useful for the treatment of opioid addiction through blockade of opioid reward. Surprisingly, however, an initial human laboratory study found that a single administration of the NK_1R antagonist aprepitant potentiated, rather than inhibited, subjective as well as physiologic responses to an opioid challenge in prescription opioid abusers (Walsh et al., 2012). A direct assessment of opioid self-administration after NK_1R blockade is therefore critical but has to date not been obtained in laboratory animals or humans. Furthermore, the role of the NK_1R in opioid-related behaviors influenced by stress, for example, stress-induced reinstatement of opioid seeking after extinction, has not been explored.

In contrast to its role in opioid-related behaviors, disruption of NK_1R signaling does not affect cocaine CPP, self-administration, or locomotor sensitization (Gadd et al., 2003; Murtra et al., 2000; Ripley et al., 2002). However, there is some evidence that NK_1R antagonists can suppress cocaine-induced locomotion (Kraft et al., 2001) and that relapse to cocaine seeking after extinction can be triggered by ICV infusion of a specific NK_1R agonist (Placenza et al., 2005) or intra-VTA infusion of an SP analog (Placenza et al., 2004). However, an NK_1R specific antagonist was unable to prevent reinstatement of cocaine seeking induced by cocaine priming (Placenza et al., 2005). One possibility is therefore that exogenous SP is able to activate pathways involved in reinstatement of cocaine seeking, but that this does not reflect actions of endogenous SP. Alternatively, cocaine-induced reinstatement may be mediated by an NK receptor other than NK_1R, such as NK_3R. Finally, it is possible that the NK_1R is involved in reinstatement of cocaine seeking triggered by some stimuli, but not that induced by drug priming. Reinstatement induced by stress is clearly a candidate here, given the role of SP/NK_1R in stress responses.

SP and NK_1Rs in Alcohol Addiction-Related Behaviors

Most recently, a series of studies has indicated that the SP/NK_1R system is involved in alcohol-related behaviors. For example, NK_1R knockout mice do not exhibit CPP for alcohol and consume less alcohol in voluntary two-bottle choice drinking (George et al., 2008; Thorsell et al., 2010). NK_1R antagonist administration in wild-type mice also decreases alcohol consumption (Thorsell et al., 2010), as does microRNA silencing of NK_1R expression (Baek et al., 2010). Additionally, the NK_1R knockout mice fail to escalate their alcohol consumption after repeated cycles of deprivation, suggesting that the SP/NK_1R may mediate neuroadaptations that contribute to escalation (Thorsell et al., 2010).

In rats that had not been selected for alcohol preference, NK_1R antagonism did not affect alcohol self-administration or two-bottle choice consumption until doses were reached that also suppressed sucrose consumption, indicating actions on appetitive behavior that were not selective for alcohol (Steensland et al., 2010). However, systemic NK_1R antagonist administration suppressed stress-induced reinstatement of alcohol seeking in nonselected rats, at doses that had no effect on baseline operant self-administration of alcohol or sucrose, cue-induced reinstatement of alcohol seeking, or novel environment-induced locomotion (Schank et al., 2011).

The ability of NK_1R antagonism to suppress stress-induced reinstatement of alcohol seeking without affecting baseline self-administration or cue-induced reinstatement is reminiscent of compounds that target the CRF_1R (Koob and Zorrilla, 2010; Shalev et al., 2010). These compounds also control escalated alcohol consumption that results from neuroadaptations induced by a history of alcohol dependence or in models in which escalation has resulted from genetic selection for alcohol preference (Heilig and Koob, 2007). In other words, these compounds are primarily effective under conditions in which the activity of stress-responsive systems has been persistently upregulated. A hypothesis that remains to be addressed is whether NK_1R antagonists, while leaving basal alcohol intake unaffected, might be able to suppress escalated alcohol consumption. It will also be important to assess whether NK_1R antagonism will be able to influence stress-induced relapse to drug seeking and escalated (as opposed to basal) self-administration of other drug classes, including opioids and cocaine.

Evaluating the Potential of SP/NK_1R System as a Therapeutic Target

Safe and well-tolerated nonpeptide, orally available, and brain penetrant NK_1R antagonists are available and have allowed initial translation of the laboratory animal findings in a human patient population (George et al., 2008). The preclinical findings have been supported by these initial human data, in which administration of an NK_1R antagonist to treatment-seeking, alcohol-dependent patients decreased alcohol craving during early abstinence. This effect was seen both under unprovoked conditions and in response to a challenge that combined exposure to a social stressor and alcohol-associated cues. This study

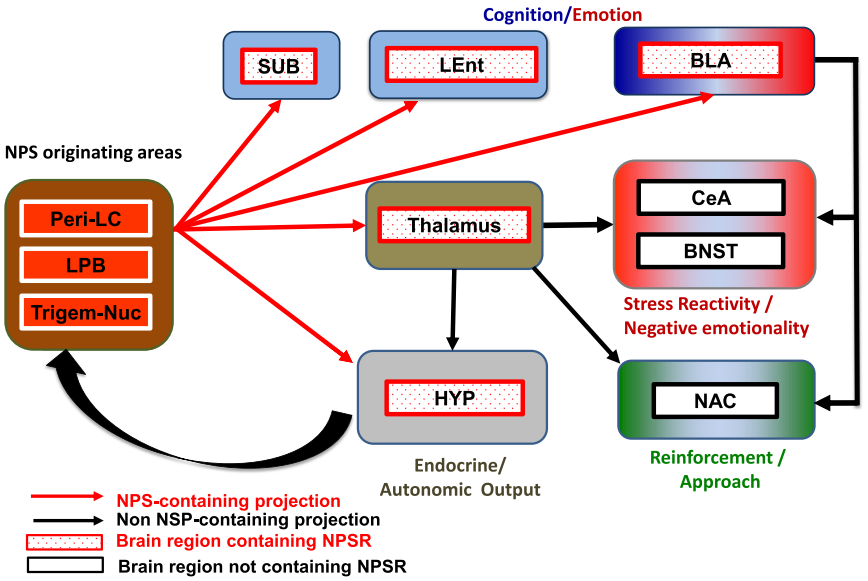

Figure 3. NPS/NPSR Circuitry Potentially Impacting Addiction-Related Behaviors

NPS is expressed in about 500 cells located between the Peri-LC, the LPB, and the principal sensory trigeminal nucleus (Trigem-Nuc), where it is largely coexpressed with glutamate and CRF, respectively. NPS cells (red arrows) project to three main target clusters: the HYP (gray), which regulates basic physiological functions such as feeding and arousal; the thalamus (light brown), which integrates somatosensory imputs, as well as endocrine and autonomic responses; and a third cluster (light blue) composed of the subiculum (SUB), BLA, and lateral entorhinal cortex (LEnt), involved in emotional memory. The thalamus sends non-NPS projections (black arrows) to several brain regions, including CeA and BNST, which are involved in emotional aspects of stress responses. The CeA as well as the NAC also receive heavy non-NPS projection from the BLA and cortical areas (not depicted in this schematic) that integrate cognitive function with emotional stress and reward processing. NPS neurotransmission is located upstream of these pathways and can therefore have complex effects on drug seeking and taking, which impact both negatively and positively reinforced aspects of these behaviors.

also demonstrated a suppression of cortisol release by the NK_1R antagonist during cue/stress exposure, suggesting a role of the NK_1R in regulation of stress-induced HPA axis function, as mentioned above. Finally, these findings were complemented by neuroimaging data, which showed that NK_1R antagonist administration potently blocked activation of stress-responsive neurocircuitry after presentation of strongly aversive visual stimuli. Subsequent genetic analyses have suggested an association of specific haplotypes within the *TacR1* locus, which encodes the NK_1R, with increased risk for alcohol dependence (Seneviratne et al., 2009). Genetically defined subgroups of patients may therefore be particularly responsive to NK_1R antagonism.

Neuropeptide S and Its Receptor

Basic Features of the NPS/NPSR System

NPS is a 20 amino acid peptide identified as the endogenous ligand for the deorphanized GPR 154, currently named the NPS receptor (NPSR) (Xu et al., 2004). In situ hybridization studies have shown that NPS precursor mRNA is expressed in about 500 cells localized only in three brainstem regions: the peri-LC area, the principal sensory trigeminal nucleus, and the lateral parabrachial nucleus (LBP) (Figure 3; Liu et al., 2011; Xu et al., 2007). A dense hypocretin/orexin fiber network surrounding NPS-positive cells has been described, suggesting the possibility of crosstalk between these two neuronal populations (Liu et al., 2011).

NPSR is G_q/G_s coupled, and its activation by NPS induces mobilization of Ca^{2+}, stimulates cAMP synthesis, and increases cellular excitability (Meis et al., 2008; Reinscheid and Xu, 2005; Xu et al., 2004; Yoshida et al., 2010). In contrast to the anatomically restricted expression of the NPS transcript, NPSR is widely expressed in the brain, including olfactory regions, the AMG complex, and other limbic structures (Leonard and Ring, 2011; Liu et al., 2011; Xu et al., 2007). The widespread distribution of the NPSR and its mRNA in the brain indicate that the NPS system may be important in regulating a variety of physiological functions.

NPS, NPSR, and Stress Responses

Activation of NPSR results in an unusual behavioral profile. On one hand, it has been shown that NPS activates arousal and stress-responsive mechanisms (Smith et al., 2006). Accordingly, and similar to CRF and other stress mediators, NPS potently decreases palatable food intake or feeding elicited by partial restriction (Beck et al., 2005; Cifani et al., 2011; Peng et al., 2010; Smith et al., 2006). However, additional studies have shown that NPS also activates the hypothalamic hypocretin/orexin system (Cannella et al., 2009; Kallupi et al., 2010; Niimi, 2006) and facilitates home-cage food consumption (Niimi, 2006). Unusually, the proarousal and prostress properties of NPS are combined with potent anxiolytic-like actions (Jüngling et al., 2008; Leonard et al., 2008; Rizzi et al., 2008; Vitale et al., 2008). Furthermore, NPS appears to reduce expression of the conditioned fear response and facilitate fear extinction through actions at extrahypothalamic sites, an effect independent from its immediate anxiolytic-like action (Jüngling et al., 2008; Meis et al., 2008).

NPS and Addiction-Related Behaviors

Neurochemical studies have suggested that central injection of NPS facilitates corticomesolimbic DA neurotransmission, a hallmark of reward (Mochizuki et al., 2010; Si et al., 2010). However, ICV NPS administration induced neither place preference nor aversion (Li et al., 2009), suggesting that NPS is devoid of direct rewarding properties. When coadministered with morphine, NPS blocked the acquisition of morphine CPP (Li et al., 2009), which might suggest that NPS can block reward from drugs of abuse, but central injection of NPS or selective antagonism of the NPSR did not influence cocaine self-administration (Kallupi et al., 2010;

Okamura et al., 2008). Genetic influences affect the impact of NPS on alcohol consumption in rats, with alcohol-preferring rat strains exhibiting decreased alcohol drinking in response to NPS (Badia-Elder et al., 2008; Cannella et al., 2009, European Behavioral Pharmacology, conference). The alcohol-preferring rat strains used in these studies are highly stress reactive and show increased measures of anxiety-like behavior. It is therefore possible that, in alcohol-preferring rats, NPS decreases alcohol consumption through its anxiolytic-like properties.

One of the most striking features of NPS pharmacology in relation to addiction is its ability to promote relapse to drug seeking. For instance, it was shown that NPS, given ICV or into the lateral hypothalamus (LH), potentiated cue-induced relapse to alcohol seeking (Cannella et al., 2009). The permissive role of NPS, given into the LH for alcohol seeking was mediated by the hypocretin/orexin system, because peripheral administration of an orexin-1 receptor antagonist completely blocked it (Cannella et al., 2009).

Other studies have also linked NPS activity to cocaine relapse. Using a drug priming procedure, it was found that ICV injection of NPS reinstated extinguished lever pressing for cocaine in mice (Pañeda et al., 2009). This effect appeared to be mediated by a downstream activation of central CRF systems, because it was prevented by administration of a CRF_1R antagonist and was absent in CRF_1R knockout mice. Notably, the anxiolytic-like effect of NPS was preserved in CRF_1R knockout mice, suggesting that this NPS property is independent of CRF_1Rs (Pañeda et al., 2009).

The facilitatory role of NPS on relapse is further supported by experiments using a conditioned reinstatement model of cocaine seeking (Kallupi et al., 2010). In this study, NPS potently reinstated relapse after ICV or intra-LH microinfusion. Administration of the NPSR antagonist SHA 68 reduced cue-induced reinstatement of cocaine seeking, supporting a role for endogenous NPS in cocaine relapse. In this system, the effect of NPS on drug relapse is mediated by downstream activation of both the hypocretin/orexin and CRF_1R systems.

Recently, a link was also proposed between the NPS system and alcohol withdrawal (Ruggeri et al., 2010). The data in this study suggest that elevated expression of NPSR after a history of alcohol dependence may represent a neuroadaptive mechanism that attempts to compensate for the increased anxiety of the animal strains used. This neuroadaptation may set the scene for a dynamic in which increased NPS neurotransmission, initially induced as a compensatory mechanism to counteract withdrawal anxiety, persists and promotes relapse during later stages of abstinence. It is also known that protracted abstinence is associated with increased HPA axis activity and higher peripheral corticosteroid levels (Rasmussen et al., 2000; Zorrilla et al., 2001). NPS given into the paraventricular nucleus increases ACTH release and augments plasma glucocorticoid levels (Smith et al., 2006), which may contribute to hormonal dysregulation occurring during the postdependent state, further contributing to relapse behavior (Sinha et al., 2011).

Evaluating the Potential of NPS/NPSR System as a Therapeutic Target

The NPS system plays a role in the regulation of several addiction-related mechanisms, in particular withdrawal (Ruggeri et al., 2010) and relapse to drug seeking (Cannella et al., 2009; Kallupi et al., 2010; Pañeda et al., 2009). Together, these data indicate that the NPS/NPSR system may represent a therapeutic target in addiction. Of particular interest is the possibility that NPSR antagonists may be useful in the treatment of drug craving and relapse. Nonpeptide NPSR antagonists that can be used as tools to probe the biology of the NPS system have been developed (Okamura et al., 2008; Patnaik et al., 2010), but none of these have properties that would render them suitable for clinical development at present state.

Conclusions and Future Prospects

Outlining a Systems-Level Organization

Appetitive, approach-promoting mechanisms are critical for the initiation phase of addiction. As addiction develops, negative emotional states triggered by stress and withdrawal promote negatively reinforced drug seeking and taking, through activity of systems that encode aversive emotional states and that have evolved to motivate behavioral avoidance. Upregulated CRF/CRF_1R function within the AMG is a key factor behind this negatively reinforced drug seeking and taking (Heilig and Koob, 2007; Koob and Zorrilla, 2010). Within the AMG, CRF and NPY oppositely influence CeA output after stress exposure (Gilpin and Roberto, 2012; Heilig et al., 1994). Stress modulators other than CRF and NPY are likely to act upstream of the CeA circuitry or interact with it to drive negatively reinforced drug seeking. The precise organization of these systems has for the most part not been studied directly, and even the limited data available are inconclusive. Clearly, we are only at the beginning of understanding the interactions within these complex networks.

As a framework for beginning to define the organization of stress-related peptide systems in relation to addiction, Figure 4 provides a schematic of neurocircuitry that drives drug seeking and taking (adapted from Koob and Volkow, 2010). Into the schematic are integrated key nodes where the modulators discussed in our Review can act to promote relapse and drug taking under stressful, aversive conditions (red colors). Some information to begin outlining this organization is available. For example, N/OFQ appears to reduce stress-induced alcohol seeking and escalated consumption through antistress actions within local CeA circuitry, where it presumably directly opposes CRF/CRF_1R actions (Economidou et al., 2008). Ucn/CRF_2R systems interact with dynorphin within the AMG but can also exert their influence at the level of the DR (Vuong et al., 2010), a structure that is activated by stress and sends serotonergic projections to both AMG and NAC. Ucn/CRF_2R activity can also modulate the activity of the LS which projects to both AMG and HYP, and whose activity promotes active stress coping and suppresses endocrine stress responses (Singewald et al., 2011). SP/NK_1Rs promote stress responses and are positioned to drive negatively reinforced drug seeking through actions at the level of the DR, LS, and AMG (Ebner et al., 2008a). Finally, release of NPS, whose activation of NPSR suppresses anxiety-like behavior (Xu et al., 2004), has recently been shown within the BLA in response to stress (Ebner et al., 2011). A further layer of complexity is added by the fact that, in addition to their stress-modulating actions, Ucn:s, SP, N/OFQ, and NPS can also influence drug seeking through

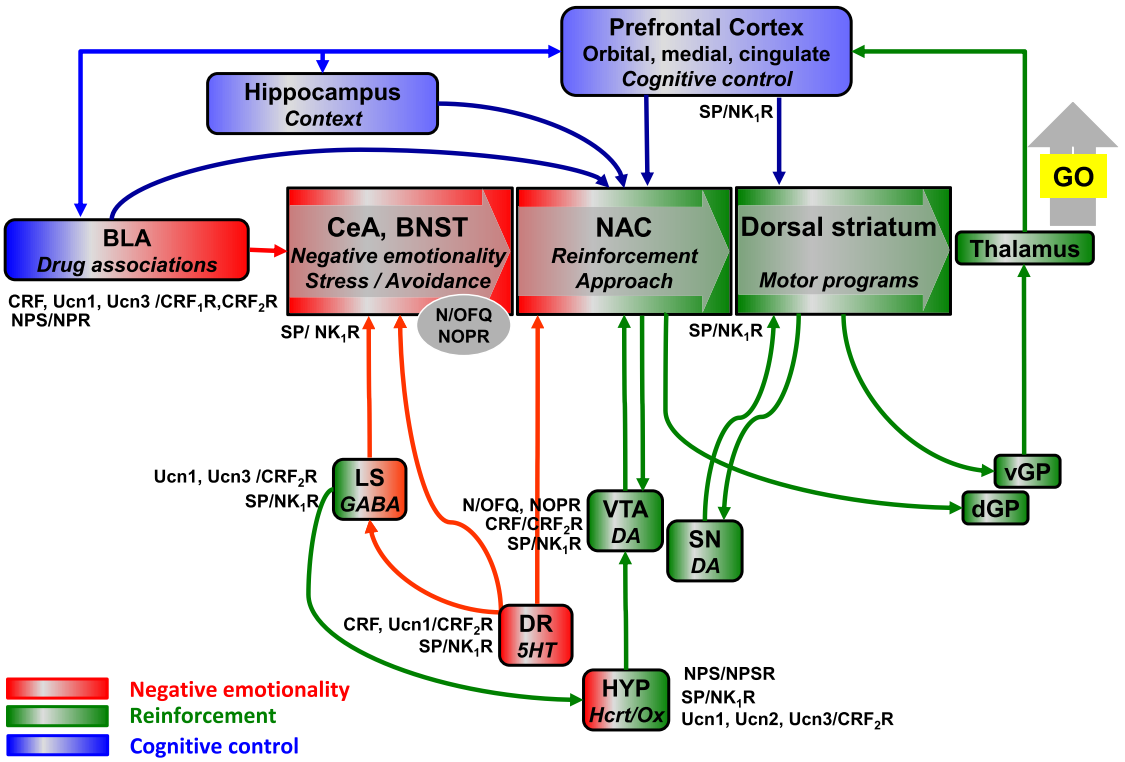

Figure 4. An Integrated View

Key nodes in circuitry that drives drug seeking and self-administration (adapted from Koob and Volkow, 2010) modulated by Ucn family peptides and CRF_2R, SP and NK_1R, NPS and NPSR, and N/OFQ and NOPR systems. Nodes at which modulation is likely to occur through effects on stress reactivity and negative emotionality are shown in red; those at which modulation is likely to influence appetitive or approach-related mechanisms are depicted in green. The figure shows that the systems discussed in this Review can impact addiction-related behaviors at multiple sites. Their impact is likely to vary with genetic factors that influence the functional activity of the respective system, as well as drug exposure history of the individual and concomitant neuroadaptations. Although many effects on drug seeking and taking have been described after manipulation of these systems, their complexity suggests that extensive research will be required to properly assess their potential as therapeutic targets and to define patient characteristics most likely predictive of efficacy (Hcrt/Ox, hypocretin/orexin; vGP, ventral globus pallidus; dGP, dorsal globus pallidus).

pathways mediating positively reinforcing drug effects (shown in green in Figure 4). Finally, emerging data indicate that the habenula (not shown in the figure), a structure that is rich in NK_1R receptors, may be at the intersection of "reward" and "antireward" pathways and negatively reinforce behavior through inputs to the VTA (Stamatakis and Stuber, 2012).

Assessing Therapeutic Potential

It is conceptually attractive to target systems that drive negatively reinforced drug seeking and taking for clinical development of therapeutics, but there are numerous challenges to realizing that potential. Technical and practical issues differ markedly between the systems. At one end of the spectrum, NK_1R antagonists with acceptable safety, tolerability, and ability to engage central targets are widely available and have enabled initial clinical trials. At the other, selective nonpeptide CRF_2R ligands are still lacking, posing challenges even for early preclinical target validation studies.

The conceptual challenges for drug development in this area are more interesting and perhaps also more challenging. First, an understanding of how these systems are organized and interact will be critical for assessing their therapeutic potential. If, for instance, Ucn:s and dynorphin signaling are indeed organized in series as proposed, with kappa opioid receptor activation downstream of Ucn/CRF_2R activity in a final common pathway of stress reactivity (Bruchas et al., 2010), then therapeutics targeting CRF_2R may have little to offer beyond those that block kappa opioid receptors, which are further along in clinical development. However, other Ucn pathways also contribute to addiction-related behaviors, leaving the possibility that additive effects may be possible.

Second, data on currently approved as well as emerging therapies suggest that individual patient factors determine sensitivity to medications targeting different peptide systems (for review, see Heilig et al., 2011). Functional genetic variation as well as environmental exposures (including drug exposure) is able to influence the functional activity of individual mediator systems. As an example, it was recently found that a functional *NPSR* polymorphism is associated with panic anxiety and autonomic reactivity to stress (Domschke et al., 2011), as well as increased BLA activation during emotional processing (Dannlowski et al., 2011). These data strongly suggest that if NPSR antagonists turn out to have a therapeutic potential in addictive disorders, their efficacy will probably vary with patient genetics at this locus. Association of variation at the *TacR1* locus that encodes the NK_1R with alcoholism suggests a similar possibility, although in that case, the functional consequences have not yet been

established. Furthermore, if the history of drug exposure influences CRF_2R signaling in a way that modulates stress reactivity, as suggested by animal data (Vuong et al., 2010), then drug exposure history may also need to be taken in account to define optimally responsive patient populations.

Concluding Comment

Motivational mechanisms that underlie escalation of drug seeking and relapse are complex and vary both between individuals and, over time, within an individual. We have reviewed recent additions to a growing number of stress-related neuropeptide modulators that, based on preclinical studies, have been suggested to contribute to drug seeking and taking. These findings hold the promise of expanding therapeutic options in addictive disorders, but the promise comes with considerable challenges. The multiple systems involved, their interactions, and the multiple levels at which they can influence behavior should serve as a warning against overly simplistic predictions of therapeutic potential. Personalized medicine approaches that take in account genetic variation in genes encoding elements of these systems, and ways in which environmental exposures (including drug exposure) influence them, will likely become critical determinants of efficacy. Basic science will be vital to determine the relative impact of genetics, environment, and drug use history to the function of each system. Once such data emerge, they will hopefully help guide clinical development.

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