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The role of the neuropeptide S system in addiction: Focus on its interaction with the CRF and hypocretin/orexin neurotransmission

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ABSTRACT

Recent behavioral, pharmacological and molecular findings have linked the NPS system to drug dependence. Most of the evidence supports the possibility that increased NPS activity may contribute to shaping vulnerability to addiction, especially relapse. However, data suggesting that the anxiolytic-like properties of NPS may have protective effects on addiction have been also published. In addition, evidence from conditioned place preference experiments, though not unequivocal, suggests that NPS *per se* is devoid of motivational properties. Intriguingly, several effects of NPS on drugs of abuse appear to be mediated by downstream activation of brain corticotrophin releasing factor (CRF) and hypocretin-1/orexin-A (Hcrt-1/Ox-A) systems. The major objective of the present article is to review the existing work on NPS and addiction. Particular attention is devoted to the interpretation of findings revealing complex neuroanatomical and functional interactions between NPS, CRF, and the Hcrt-1/Ox-A systems. Original data aimed at shedding light on the role of NPS in reward processing are also shown. Finally, existing findings are discussed within the framework of addiction theories, and the potential of the NPS system as a treatment target for addiction is analyzed.

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Abbreviations: NPS, neuropeptide S; NPSR, neuropeptide S receptor; CRF, corticotrophin releasing factor; Hcrt-1/Ox-A, hypocretin-1/orexin-A; GPR154, G protein coupled receptor 154; ppNPS, prepropeptide NPS; Lys–Arg, lysine–arginine; mRNA, messenger RNA; LC, locus coeruleus; PeF, perifornical (area); EGFP, enhanced green fluorescent protein; KF, Kölliker–Fuse; GPCR, G protein coupled receptor; cAMP, cyclic adenosine mono phosphate; hNPSR-A, human NPSR form A; hNPSR B_{LONG}, human NPSR form B; VMH, hypothamalic ventromedial nucleus; DMH, dorsomedial hypothalamic nucleus; ICV, intracerebroventricular; LA, lateral amygdala; BLA, basolateral amygdala; EPM, elevated plus maze; PaV, paraventricular nucleus; ACTH, adrenocorticotropic hormone; AVP, arginine-vasopressin; EPN, endopiriform nucleus; PFC, prefrontal cortex; VTA, ventral tegmental area; DA, dopamine; GABA, Gamma aminobutyric acid; CPP, conditioned place preference; msP, Marchigian Sardinian (alcohol) preferring (rats); LH, lateral hypothalamus; OX_1, orexin 1 receptor; NMDA, N-methyl-D-aspartic acid; NA, noradrenaline; BNST, bed nucleus of stria terminalis; HPA, hypothalamic pituitary adrenal; CRF_1, CRF receptor 1; WT, wild type; KO, knock out; IP, intraperitoneal; CeA, central amygdala; LV, lateral ventricle; ACo, anterior cortical amygdaloid nucleus; M2, motor cortex; EN, endopiriform nucleus.

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1. Introduction

Neuropeptide S (NPS) is a 20 amino-acid peptide identified as the endogenous ligand for the deorphanized G-protein coupled receptor 154 (GPR 154), currently named the NPS receptor (NPSR) (Xu et al., 2004). This name was coined since the N-terminal residue, is a serine that is conserved in all species. The primary structure of NPS is highly conserved in higher vertebrates, but seems to be absent in fish (Reinscheid, 2007). NPS results from the cleavage of a prepropeptide (ppNPS) that shares the typical structural characteristics of other neuropeptide precursors. The cleavage of the precursor at a proteolytic processing site constituted by a Lys–Arg residue pair, releases the 20 amino acid mature peptide (Xu et al., 2007).

The NPS precursor gene is encoded by three small exons interrupted by two introns. The genomic organization of the NPS precursor gene seems to be identical in all species; only a few positions in the center and in the carboxy-terminal part of the peptide show sequence variations, indicating that the amino-terminus might contain structures critical for receptor binding (Reinscheid, 2007).

Detailed *in situ* hybridization studies have shown that ppNPS mRNA is expressed in only three rat brainstem regions; the peri-locus coeruleus (LC) area, the principal sensory trigeminal nucleus, and the lateral parabrachial nucleus (Xu et al., 2007). Confirming this finding, in a recent work using transgenic mice that express enhanced green-fluorescent protein (EGFP) under control of the endogenous ppNPS promoter, it was shown that there are about 500 NPS cells in the brainstem; all are localized between the Kölliker–Fuse nucleus (KF) of the lateral parabrachial area and the peri-LC area (Liu et al., 2011).

In the peri-LC area, NPS expressing neurons are predominantly glutamatergic, with a few cholinergic cells found in the lateral portion of this structure. In the principal sensory trigeminal nucleus, NPS co-localizes with glutamate, while in the lateral parabrachial nucleus, co-expression with CRF has been reported (Xu et al., 2004, 2007). Of note, in the EGFP mice a dense orexin/hypocretin fiber network surrounding NPS positive cells was described, thus suggesting the possibility of cross-talk between these two neuronal populations (Liu et al., 2011).

The NPSR has the typical consensus GPCR structure, with seven trans-membrane domains. In humans, the NPSR gene encodes at least eight receptor variants, but evidence suggests that only two of them, hNPSR-A and hNPSR B_{LONG}, that differ in their C-termini, produce functional receptors expressed on the cell membrane (Vendelin et al., 2005). The receptor variant hNPSR-A was found in two forms given by an A/T single-nucleotide-polymorphism (SNP) at position 107 coding for an Asn-Ile exchange (Laitinen et al., 2004). NPS showed a 10-fold higher potency on the NPSR Ile^{107} variant compared to NPSR Asn^{107} stimulating a higher mobilization of intracellular Ca^{2+} and cAMP formation (Reinscheid et al., 2005). Evidences demonstrate that the Asn-Ile^{107} SNP is related to panic disorders. In panic disorder (PD) patients the A/A genotype, encoding for NPSR Asn^{107}, was under-represented whereas both heterozygous (A/T) and homozygous (T/T) Ile^{107} variants were increased compared to healthy controls (Domschke et al., 2011; Okamura et al., 2007).

In rodents, only one variant of NPSR has been found, exhibiting high sequence homology with hNPSR-A (Pulkkinen et al., 2006). NPSR couples to both Gq and Gs proteins, hence receptor 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).

Contrary to NPS, NPSR mRNA is widely expressed in many brain regions, including the olfactory regions, amygdala complex, and other limbic structures. NPSR mRNA is also expressed in the input and output regions of the hippocampal formation, and in multiple hypothalamic regions, such as the arcuate nucleus, hypothalamic ventromedial nucleus (VMH), and dorsomedial hypothalamic nucleus (DMH) (Liu et al., 2011; Xu et al., 2007).

Recently, the availability of specific antibodies has allowed analysis of the distribution of the NPSR protein in rat brain. Results revealed that the distribution of NPSR protein is consistent with the distribution of NPSR mRNA (Leonard and Ring, 2011). The widespread distribution of the NPSR and its mRNA in the brain indicates that the NPS system may be important in regulating a variety of physiological functions.

A seminal work by Xu et al. (2004) demonstrated that intra-cerebro-ventricular (ICV) injection of NPS increased wakefulness and reduced all stages of sleep in rats. In the same study it was shown that in mice, NPS stimulated locomotion in both naïve and habituated animals, and exerted an anxiolytic-like effect in a variety of behavioral tasks (*i.e.*, marble burying, elevated plus-maze, light-dark box, open field). These findings have been confirmed in follow-up studies, where the striking anxiolytic/pro-arousal traits of NPS were not only confirmed but expanded using a larger battery of behavioral tests, including the four-plate test and stress induced hyperthermia (Jungling et al., 2008; Leonard et al., 2008; Rizzi et al., 2008; Vitale et al., 2008). Interestingly, ICV as well as site specific injection of NPS into the lateral amygdala (LA) and baso-lateral amygdala (BLA) exerted an anxiolytic-like effect in the elevated plus-maze (EPM), light dark box, and open field, but did not increase locomotor activity (Jungling et al., 2008; Leonard et al., 2008; Meis et al., 2008). A role for NPS in the modulation of stress and arousal has also been documented, following ICV and intra-hypothalamic paraventricular nucleus (PaV) administration studies in which it was shown that NPS activates locomotion, increases plasma ACTH and corticosterone levels, and induces CRF and arginine-vasopressin (AVP) release (Smith et al., 2006). In another study, it was shown that ICV injection of NPS activates hypocretin-1/orexin-A (Hcrt-1/Ox-A) neurons in the hypothalamus (Niimi, 2006).

The NPS system has been shown to be involved in the modulation of conditioned fear behavior in mice (Jungling et al., 2008; Meis et al., 2008). Injection of NPS into the endopiriform nucleus (EPN) reduced freezing behavior during contextual fear memory retrieval, an effect probably obtained through activation of the BLA *via* stimulation of glutamatergic synaptic activity (Meis et al., 2008). Injection of NPS in the same area did not affect anxiety behavior in the EPM, suggesting that activation of NPSR in the EPN selectively modulates fear conditioned memory but is not involved in the modulation of general anxiety (Meis et al., 2008). In addition, when administered into the LA and BLA, NPS reduced expression of the conditioned fear response and facilitated fear extinction (Jungling et al., 2008; Meis et al., 2008).

The NPS possesses a unique pharmacological profile being able to mediate dissimilar and to a large extend opposite physiological actions such as arousal and anxiolysis. Unfortunately the data available so far do not allow a conclusive explanation for this dualistic role of NPS, but few speculations are possible.

Several findings demonstrate that NPS has an important neuromodulatory role and its activation is generally associated with increased CRF_1 and Hcrt-1/Ox-A receptor-1 (OX_1) activity (Cannella et al., 2009a; Kallupi et al., 2010; Paneda et al., 2009), and stimulation of excitatory neurotransmission mediated by glutamate (Meis et al., 2008; Xu et al., 2007). This provides a neurochemical background supporting the role of this system in the modulation of pro-arousal functions, stress, locomotion, memory and vigilance.

On the other hand, NPS decreases fear expression and facilitates fear extinction by means of mechanisms involving increased inhibitory inputs to the CeA (Jungling et al., 2008). According to recent findings CRF activates NPS expressing neurons in the brainstem. This could be part of a feed back mechanism where increased release of NPS in terminal areas (*i.e.*, the CeA) could serve to control emotional reactivity to stress thus facilitating engagement into stress-coping behaviors (Jungling et al., 2012).

An intriguing hypothesis to reconcile this dualistic nature of NPS is that increased arousal and vigilance together with attenuated fear and anxiety may result in a physiological state aimed at enhancing the chances to emit effective responses to threatening events. The NPS system might have evolved to respond to this need.

Several studies have demonstrated that NPS is able to play a role in the modulation of food intake in rodents. ICV NPS injection transiently reduced food intake in fasted rats (Beck et al., 2005). These data were then replicated by Smith and colleagues after intra-PaV injections of the peptide (Smith et al., 2006). The anorexigenic effect of NPS has been confirmed in different models such as fasted mice and food restricted rats, as well as in freely feeding animals fed with standard or palatable food diets (Cifani et al., 2011; Peng et al., 2010). In all these cases, the NPS anorectic effect was antagonized by the NPSR antagonists, confirming that this effect is mediated by the NPSR. The PaV appeared to be the site of action in the brain for the anorexic effects of NPS (Fedeli et al., 2009).

Robust behavioral, pharmacological, and molecular findings have also linked the NPS system with drug addiction. Most of these

Table 1
Studies directly addressing the role of NPS in drug addiction.

Drugs of abuse	Experimental procedure	Pharmacological tools	Route of administration	Brain area	Key findings	Reference
Cocaine	Self-administration	NPS SHA 68, NPSR-QA_1	ICV IP	LV Peripheric	No effect	Kallupi et al. (2010)
	Cue-induced reinstatement	NPS	Site specific injection	LV, LH, PeF, DMH	Increase cue-induced reinstatement	
		SB-334867	IP	Peripheric	Block NPS effect on cue-induced reinstatement	
		SHA 68 NPSR-QA_1			Decrease cue-induced reinstatement	Kallupi et al. (2012)
		[D-Cys(tBut)5]NPS	Site specific injection	LH, PeF		
	Cocaine place conditioning	NPS	ICV	LV	No effect	Cannella et al. (original communication), present manuscript, Figs. 3 and 4
	Reinstatement of extinguished place preference	NPS	ICV	LV	No effect	
	NPS-induced reinstatement	NPS	ICV	LV	Reinstate cocaine seeking	Paneda et al. (2009)
		Antalarmin	IP	Peripheric	Decrease NPS-induced reinstatement	
		NPS/CRF1R KO	ICV	LV	No effect	
	Marble burying, light-dark transfer	NPS/CRF1R KO	ICV	LV	Decrease anxiety	
Amphetamine	Locomotor activity	NPS	Site specific injection	Cortex, cerebellum, striatum	Increase locomotor activity	Castro et al. (2009)
Caffeine	NPS/NPSR gene expression	Caffeine (chronic)	IP	Hypothalamus	Increase NPSR expression	Lage et al. (2006)
				Brainstem	No effect	
Nicotine	NPS/NPSR gene expression	Nicotine (chronic)	SC	Hypothalamus, brainstem	Increase NPS/NPSR gene expression	Lage et al. (2007)
Alcohol	NPSR gene expression	Alcohol	Per os	M2, ACo, BLA, En, LH, CeA, PaV	Increase NPSR gene expression	Ruggeri et al. (2010)
	Defensive burying	NPS	ICV	LV	Decrease anxiety	
	Self-administration	NPS	ICV	LV	No effect	Cannella et al. (2009a,b)
	Cue-induced reinstatement	NPS	Site specific injection	LH	Increase cue-induced reinstatement	
		SB-334867	IP	Peripheric	Block NPS effect on cue-induced reinstatement	
	Ethanol intake	NPS	ICV	LV	No effect	Badia-Elder et al. (2008)
Morphine	Conditioned place preference	NPS	ICV	LV	Block morphine-induced conditioned place preference	Li et al. (2009)
	Locomotor activity	NPS	ICV	LV	No effect on morphine-induced hypolocomotion Increase locomotor activity	

studies support the possibility that increased NPS activity may play a role in shaping vulnerability to addiction; especially to relapse. However, data suggesting that the anxiolytic-like properties of NPS may have protective effects on addiction have also been published. Moreover, the absence of effects on self-administration of a drug of abuse following manipulation of the NPS system renders the role of this peptide system in addiction intriguing (Table 1).

Various studies were performed using NPSR knock out (−/−) mice in the attempt to analyze the biological functions controlled by NPS system especially anxiety, locomotion and arousal. However, in relation to these traits no major phenotypic differences between NPSR (−/−) and wild type (WT) mice have been so far evidenced. The results of three distinct studies carried out with NPSR (−/−) mice derived by C57BL/6 strain showed that there were no difference in anxiety between NPSR (−/−) and WT animals (Fendt et al., 2011; Ruzza et al., 2012; Zhu et al., 2010). One exception is represented by NPSR (−/−) derived by 129S6/SvEv strain that showed a hyper-anxious phenotype when compared to NPSR WT in EPM, light-dark box and open field (Duangdao et al., 2009). The 129S6/SvEv mice line shows basal anxiety levels higher than C57BL/6 mice. It has been proposed, therefore, that in this former line the NPS/NPSR system may be hyperfunctioning to compensate for the innate higher anxious-like trait of these animals (Ruzza et al., 2010). Receptor knock down may therefore more likely result in a phenotype in 129S6/SvEv than in C57BL/6 background. To date, no specific studies have been carried out using mice lacking NPSR to investigate drug addiction related behaviors.

The major objective of the present article is to review the existing work on NPS and addiction. Original data aimed at shedding light on the role of NPS in reward processing are also shown. Finally, existing findings are discussed within the framework of addiction theories, and the significance of the NPS system as a treatment target for addiction is analyzed.

2. NPS and neurotransmitters

In the LC, NPS co-expresses with the excitatory neurotransmitter glutamate, while in the parabrachial nucleus it co-localizes with CRF (Xu et al., 2007) and glycine (Liu et al., 2011; Xu et al., 2007). Several reports have indicated that NPS increases glutamatergic signaling in the amygdala, one of the most important brain areas involved in emotional processing (Jungling et al., 2008; Meis et al., 2008). At the functional level, this may reflect the modulatory effects of NPS on fear related information processing, anxiety states, and possibly addiction. Indirect evidences for an interaction between NPS and glutamatergic transmission are also provided by data showing that central administration of the peptide reduces spatial memory impairment produced by the N-methyl-D-aspartate receptor antagonist MK801 (Han et al., 2009). In another study it was also shown that treatment with NPS allowed mice to readily recover from the disruption of pre-pulse inhibition caused by MK801 treatment (Okamura et al., 2010).

Only a few neurochemical studies have been carried out so far to investigate the effects of NPS on release of other neurotransmitters. In recent microdialysis experiments, it was shown that central administration of NPS increased extracellular dopamine levels in the prefrontal cortex (PFC) (Si et al., 2010). Another group found that intra VTA injection of NPS stimulated locomotor activity and increased extracellular levels of DA metabolites in the nucleus accumbens shell (Mochizuki et al., 2010). These observations suggest a role for the NPS system in the regulation of the corticomesolimbic DA activity, and therefore in the modulation of emotional processing, memory, and addiction (see for review: Di Chiara, 1995; Spanagel and Weiss, 1999). Recent studies have analyzed the effect of NPS on serotonin neurotransmission. Using synaptosome preparations from the mouse medial prefrontal cortex, it was shown that picomolar concentrations of NPS inhibited the release of serotonin (Raiteri et al., 2009). This observation was made despite the fact that frontal cortex exhibits only a low level of expression of NPSR (Xu et al., 2007). On the other hand, these results were not replicated by *in vivo* microdialysis experiments, as they showed that central administration of NPS did not change extracellular serotonin levels (Si et al., 2010). Additional studies are required to clarify the reasons for these discrepancies.

Evidence that NPS also interacts with the noradrenergic system arises from studies on the role of NPS in memory. NPS has a role in long-term memory formation, as demonstrated in inhibitory avoidance and object recognition paradigms. Moreover, administration of SHA68, an NPSR antagonist, attenuated the inhibitory avoidance memory improvement elicited by NPS; whereas NPSR (−/−) mice showed memory impairment both in the inhibitory avoidance and object recognition paradigms (Okamura et al., 2011). Since beta-adrenergic receptors contribute to memory consolidation (Introini-Collison et al., 1992; Quirarte et al., 1997), propranolol, a beta-receptor antagonist, was used to examine the interactions between NPS and adrenergic neurotransmission. The results showed that propranolol prevented NPS-induced memory improvements, thus demonstrating that the interaction between NPS and the adrenergic system is important for memory processing (Okamura et al., 2011). However, it must be noted that the interaction between NPS and noradrenaline is not necessarily direct. Neurotransmitters and neurohormones such as GABA and/or glucocorticoids which are known to co-operate with noradrenalin in memory consolidation through mechanisms involving the amygdala (McGaugh, 2004) are also recruited by NPS action (Meis et al., 2008; Smith et al., 2006) and can indirectly mediate the interaction between NPS and noradrenergic system reported by Okamura and colleagues.

A recent study investigated the contribution of the adenosine system to the hyperlocomotion induced by NPS. NPS-induced locomotion was tested in mice co-administered with either caffeine (which acts as a non-selective adenosine receptor antagonist), or with a selective antagonist for either A1 or A2A receptors. Results showed that the A2A receptor antagonist as well as caffeine reduced the increase in locomotion evoked by NPS, whereas the A1 receptor antagonist had a facilitatory role in NPS-induced locomotion. According to this result the authors proposed that A2A receptor-mediated mechanisms might play a role in NPS-induced hyperlocomotion, while the facilitatory effect showed by A1 antagonism is more likely due to an additive effect than to a real interaction between A1 and NPS system (Boeck et al., 2010).

Adenosine levels are elevated upon exposure to drugs of abuse, and adenosine A2A and dopamine D2 are known to form heterodimers that compared to the two homomeric receptors, have distinct functional properties (Brown and Short, 2008; Hack and Christie, 2003). Blockade of A2A receptors increases propensity to use drugs of abuse, while agonism of these receptors results in inhibition of drug intake (Micioni Di Bonaventura et al., 2012). A2A receptors have also been shown to modulate glutamate and cannabinoid receptors activity in the brain. (Lerner et al., 2010; Martire et al., 2011) hence it is possible that the interaction between the NPS and adenosine may, at least to some extent, contribute to shape the effect of NPS on drugs of abuse.

Finally recent pharmacological studies have revealed that NPS may function as an upstream modulator of the brain's CRF and Hcrt-1/Ox-A systems, two important mediators of drug abuse related behaviors, particularly relapse. Notably, the facilitating role of NPS in the reinstatement of drug seeking is prevented by administration of selective CRF_1 or OX_1 antagonists (Cannella et al., 2009a; Kallupi et al., 2010; Paneda et al., 2009).

3. NPS and reward

Neurochemical studies have revealed that central injection of NPS facilitates corticomesolimbic DA neurotransmission (Mochizuki et al., 2010; Si et al., 2010). Therefore, it is possible that NPS may possess rewarding properties *per se*. This physio-pharmacological aspect of the peptide was recently addressed in a conditioned place preference (CPP) study conducted in our laboratory. Using an unbiased procedure, we evaluated the ability of ICV NPS to evoke CPP in the rat. The results revealed that, under our experimental conditions, cocaine led to a marked CPP, while NPS given at three different doses (0.3, 1, and 2 nmol/rat) did not show an effect (Fig. 1). Similar results were obtained by Li and co-workers, also using an unbiased procedure, who found that NPS (0.3–10 nmol) induced neither place preference nor aversion (Li et al., 2009).

Interestingly, Li and colleagues reported that NPS co-administered with morphine blocked the acquisition of CPP elicited by opioid administration. Altogether these results indicate that NPS is devoid of rewarding properties, and that it may possibly block reward elicited by drugs of abuse. However, this latter possibility is not supported by results obtained in self-administration studies, in which it was shown that NPS administration did not modify operant responses for cocaine or for alcohol (Cannella et al., 2009a; Kallupi et al., 2010).

Unexpectedly, different results were obtained in a recent study in which a dualistic action of NPS in the place conditioning paradigm was observed. At a low dose (0.1 nmol) NPS elicited place aversion, while at a high dose (1 nmol) it induced preference (Cao et al., 2011). In the same study, it was also shown that rats learned to self-administer NPS intracranially. The self-infusion rate was reduced by the dopamine receptor antagonist SCH 23390, and by the OX_1 antagonist SB-334867. Altogether these findings point to the possibility that NPS, at least at some doses, may stimulate reward. On the other hand, the results of Cao et al. (2011) could also have alternative explanations. In the intracranial self-administration study, the rate of NPS self-infusion was rather low, although statistically significant if compared to its vehicle. In addition, the self-administration was assisted by visual cues contingently paired to lever pressing. Therefore, it cannot be excluded that the relatively modest increase in the response rate was a consequence of NPS' ability to facilitate sign tracking behavior, rather than to reward stimulation. This alternative explanation could be viewed as consistent with the pro-cognitive and vigilance enhancing properties of the peptide (Jungling et al., 2008; Meis et al., 2008).

Based on this evidence, it is difficult to draw clear conclusions on the role of NPS in reward. However, considering that in two separate studies NPS was neutral in the place conditioning paradigm, and considering that in drug self-administration studies central NPS injection did not shift operant responses for cocaine or alcohol (see below), it is tempting to speculate that NPS is devoid of motivational properties *per se*.

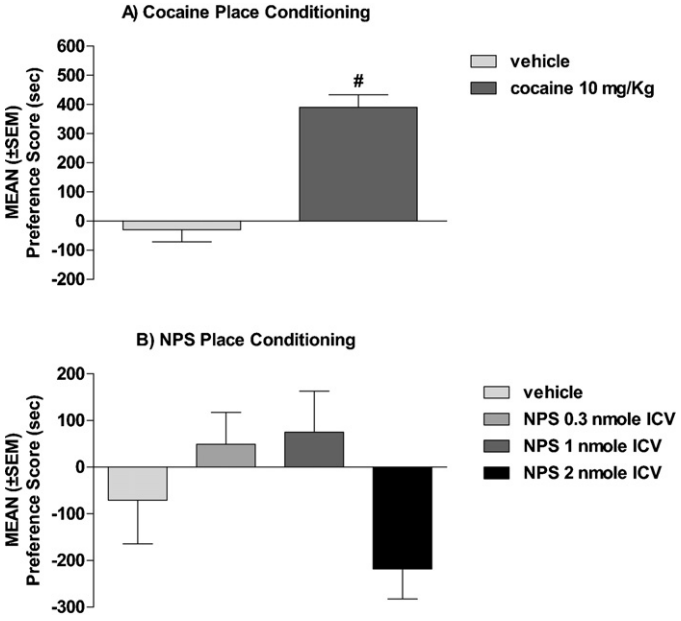

Fig. 1. NPS place conditioning. (A) Validation of conditioning model by cocaine place conditioning. Cocaine place conditioning was measured in a two compartment apparatus (different tactile and visual stimuli for each compartment) using an unbiased schedule of conditioning. Rats (n = 8/group) underwent two conditioning sessions per day for three consecutive days. The cocaine group received one daily injection of cocaine (10 mg/kg intra-peritoneally) in one compartment, and an equivalent volume of saline in the opposite, while the vehicle group received two daily saline injections. Treatments were balanced across chambers and time. On the 4th day, drug free animals were allowed to explore the entire apparatus for 15 min; time spent in each chamber was recorded by an operator unaware of the treatment conditions. Preference scores were expressed as mean ± SEM of time (in seconds) spent in the drug paired compartment minus time spent in the saline paired compartment. The vehicle group was scored as time spent in one random compartment minus the other. One between factors ANOVA revealed that cocaine treatment induced conditioned place preference [$F(1,14) = 35.20$; $p < 0.0001$]. (B) NPS place conditioning. Rats (8–9/group) underwent the same unbiased schedule of conditioning described for cocaine. The NPS group received one daily injection of NPS (0.3, 1 or 2 nmol intracerebroventricularly (ICV) in one compartment, and one of saline in the opposite, while the vehicle group received two daily saline injections. One between factors ANOVA revealed that NPS treatment did not induce development of place conditioning [$F(3,29) = 2.84$; $p > 0.05$], showing that NPS is devoid of motivational properties *per se*.

4. NPS and alcohol

Alcoholism is a chronic relapsing disorder characterized by a lack of control in limiting alcohol intake, despite adverse consequences such as negative impact on general patient health and social problems. Recent studies have provided robust evidence for implication of the NPS system in modulating the main aspects of alcohol addiction, such as intake, drug-seeking, and withdrawal (Badia-Elder et al., 2008; Cannella et al., 2009a; Ruggeri et al., 2010).

4.1. Role of NPS in alcohol reward and intake

Badia-Elder et al. (2008) demonstrated, in a two bottle choice paradigm, that central administration of NPS decreased alcohol drinking in alcohol-preferring (P) rats but not in the non-preferring (NP) control line. Similar results were obtained in our laboratory, where we found that NPS did not affect alcohol self-administration in Wistar rats (Cannella et al., 2009a), while it decreased operant responses to alcohol in Marchigian Sardinian alcohol preferring (msP) rats (Cannella et al., 2009b). The P and the msP rats are both characterized by an innate anxious phenotype; it has been hypothesized that their excessive ethanol drinking is driven in part by ethanol's ability to relieve them from this negative affective state (Ciccocioppo et al., 2006; Hansson et al., 2006; Stewart et al., 1993). Of note, Badia-Elder and co-workers also noted that at the same doses effective on alcohol intake, NPS increased the time spent in the center of an open field square without altering the total distance traveled, reflecting the possibility that in the P rats the peptide has anxiolytic-like effects. In the NP line, open field performances were not changed by NPS (Badia-Elder et al., 2008). The anxiolytic-like properties of NPS have been documented in a number of other studies, carried out in different laboratories and under various experimental conditions (Jungling et al., 2008; Leonard et al., 2008; Lukas and Neumann, 2012; Rizzi et al., 2008;

Vitale et al., 2008; Wegener et al., 2011; Xu et al., 2004); hence it is possible that, in alcohol preferring rats, NPS decreases ethanol consumption not because it acts on reward mechanisms, but rather because of its anxiolytic-like properties.

An alternative but rather unlikely hypothesis is that the effects of NPS on drinking alcohol are secondary to its anorectic actions (Beck et al., 2005; Fedeli et al., 2009; Smith et al., 2006). In fact, for P and msP rats, alcohol represents an important source of their daily caloric intake (Ciccocioppo et al., 2006). Thus the fast-inducing properties of NPS could, in theory, be responsible for a decrease in alcohol intake. However, in Badia-Elder's study (2008), it was reported that NPS elicited a moderate, even though not statistically significant, increase in food consumption in P rats. On the one hand, this fits with the view that ethanol constitutes an important source of calories for alcohol preferring rats; and in fact, the decreased ethanol-derived caloric intake was balanced by an increase in food consumption. On the other hand, it provides a strong indication that the effects of NPS on alcohol intake were not an epiphenomenon associated with peptide-induced anorexia.

4.2. Role of NPS in reinstatement of alcohol seeking and relapse

One major factor contributing to the resumption of drug use after a period of abstinence is re-exposure to environmental stimuli that have previously been associated with drug consumption (O'Brien et al., 1998; Sinha et al., 2000; Sinha and Li, 2007). Recently, we demonstrated that NPS, given ICV or into the lateral hypothalamus (LH), potentiated reinstatement of ethanol seeking induced by environmental stimuli previously paired with ethanol availability (Cannella et al., 2009a). The effect of NPS was specific for ethanol, and was not observed following re-exposure to cues predictive for water. In addition, as assessed in a repeated conditioned relapse model (Ciccocioppo et al., 2001), the action of NPS was maintained throughout consecutive reinstatement tests without development of tolerance or loss of sensitivity (Cannella et al., 2009a). The permissive role of NPS, given into the LH, on ethanol seeking, was mediated by the Hcrt-1/Ox-A system, because peripheral administration of the OX_1 receptor antagonist SB334867 completely blocked it (Cannella et al., 2009a).

The coexistence of a facilitating effect of NPS on relapse mediated by the Hcrt-1/OX-A system, and the absence of peptide effects on ethanol self-administration in Wistar rats, as well its inhibitory effect on alcohol drinking in alcohol preferring rats (reviewed above), is somewhat controversial. In fact, both in Wistars and in alcohol preferring rats, activation of the Hcrt-1/Ox-A system has been linked not only to reinstatement but also to alcohol intake (Dayas et al., 2008; Lawrence et al., 2006). For example, it was reported that activation of orexin receptors increased alcohol intake, while antagonism of orexin receptors reduced it (Lawrence et al., 2006; Moorman and Aston-Jones, 2009; Richards et al., 2008; Schneider et al., 2007). If the effects of NPS on alcohol were dependent upon its ability to activate the Hcrt-1/Ox-A system, one would expect to observe increases in alcohol drinking and relapse occurring at the same time, and in both alcohol preferring and non-selected rats. This expectation stems from the assumption that NPS may interact with Hcrt-1/Ox-A mechanisms in the ventral tegmental area (VTA); leading to potentiation of mesolimbic DA activity which in turn may be responsible for the modulation of relapse behavior and alcohol-intake (Gonzales et al., 2004; Hyman and Malenka, 2001; Le and Shaham, 2002; Spanagel and Weiss, 1999).

Indeed, it is known that despite most of the Hcrt-1/Ox-A fibers in the VTA being axons which pass to caudal brainstem structures, they can form sparse synaptic contacts with DA cells (Balcita-Pedicino and Sesack, 2007). In this area, activation of Hcrt-1/Ox-A receptors leads to an increase in NMDA receptor-mediated activity and facilitates drug of abuse-induced DA neurotransmission (Borgland et al., 2009; Espana et al., 2011). Finally, stimulation of Hcrt-1/Ox-A neurotransmission into the VTA promotes drug intake, and seems to be critical for conditioned reinstatement of drug seeking (Espana et al., 2011; James et al., 2011).

However, contrary to expectations built on this mechanism which would predict a permissive role for NPS in both relapse and alcohol drinking, it was shown that NPS facilitated relapse but either did not modify (in Wistars) or actually reduced (in P and msP rats) alcohol drinking (Badia-Elder et al., 2008; Cannella et al., 2009a,b).

To reconcile this contradiction, it could be hypothesized that NPS activates subpopulations of Hcrt-1/Ox-A neurons that do not project to the VTA. In this respect, there was a recent interesting observation that LH neurons activated by context-induced reinstatement are not those projecting to the VTA (Hamlin et al., 2008). At the neurocircuitry level, few alternative speculations can be proposed to explain how the NPS and Hcrt-1/Ox-A systems may interact to shape relapse behavior without affecting drug intake.

Perhaps the most intriguing hypothesis is the one linking the effects of these peptides to LC function. The LC receives projections from Hcrt-1/Ox-A neurons, which make synaptic contacts with noradrenergic (NA) neurons (Espana et al., 2005; Hagan et al., 1999; Horvath et al., 1999). The LC–NA system is important for selectively processing information concerning reward expectancy and the incentive value of rewards-predictive cues (Bouret and Sara, 2004, 2005); its activity is central to the evaluation of reward contingency in response to stimuli associated with emotional responses, or requiring increased vigilance (*i.e.*, alcohol cues); but not in response to non-salient conditioned stimuli (Bouret and Sara, 2004; Rasmussen and Jacobs, 1986). In line with the potential involvement of this Hcrt-1/Ox-A LC–NA circuitry in NPS effects, we found that stimulation of NPSR selectively increased lever pressing elicited by cues predictive of addictive drug reward, but not of non-rewarding solutions (Cannella et al., 2009a; Kallupi et al., 2010).

Other possibilities can be also proposed to explain the interactions between the NPS and the orexin systems. For instance, neuronal networks involving the PaV of the hypothalamus and bed nucleus of the stria terminalis (BNST) are known to express relatively abundant levels of OX_1 receptor mRNA and protein (Hervieu et al., 2001; Marcus et al., 2001). Several lines of evidence indicate a key role for these nuclei in the modulation of relapse behavior maintained by stress and by cues (Buffalari and See, 2011; Erb and Stewart, 1999; Rodaros et al., 2007). More importantly, in our laboratory we recently found that blockade of OX_1 receptors in these two areas prevented the permissive role of NPS in relapse (Cannella et al., 2010). Further studies are required to unravel the exact neuronal networks subserving NPS–orexin interactions in addiction.

4.3. NPS and alcohol withdrawal

Withdrawal syndrome is critical for the development of an addictive state. Withdrawal is characterized by both physical and psychological signs, which are strongly correlated with relapse to compulsive drug intake (Bokstrom et al., 1991; Roelofs, 1985; Roelofs and Dikkenberg, 1987; Willinger et al., 2002). Recently, we demonstrated a link between the NPS system, and alcohol intoxication and withdrawal in the rat (Ruggeri et al., 2010). *In situ* hybridization results revealed that 12 h and one week after completion of a five-day intoxication cycle, it was possible to observe an increase in NPSR mRNA in post-dependent as compared to non-dependent rats. Somatic withdrawal signs were observed at 12 h, while one week after completion of the intoxication cycle, post-dependent rats showed an increase in anxious behaviors as measured in the defensive burying test. The anxiolytic-like effect of

NPS was more pronounced in post-dependent rats than in the controls (Ruggeri et al., 2010). Altogether, these data may suggest that elevated expression of NPSR following a history of alcohol intoxication may result from an attempt to compensate for the increased anxiety occurring in these animals.

In drug addicts the risk to relapse is particularly high during withdrawal (Bokstrom et al., 1991; Roelofs, 1985; Roelofs and Dikkenberg, 1987; Willinger et al., 2002). Under these circumstances, resumption of drug use is predominantly guided by the attempt to self-medicate from the negative symptoms associated with acute (but also protracted) withdrawal. However, considering the important role of NPS in relapse, it is tempting to hypothesize that the increase in NPS neurotransmission (possibly occurring as a compensatory mechanism to counteract anxiety associated with drug withdrawal) may then contribute to shape relapse behavior during 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). Since NPS given into the PaV increases ACTH release and augments plasma glucocorticoid levels (Smith et al., 2006), it is possible to speculate that facilitation of NPS action in the PaV may contribute to this hormonal dysregulation occurring at the post-dependent state; this may also contribute to shaping relapse behavior (Sinha et al., 2011).

5. NPS and psychostimulants

To date, little is known about the role of the NPS system in the regulation of psychostimulant-related behaviors. However, some considerations on the effects of NPSR stimulation and inhibition on cocaine-induced reward and relapse to drug seeking are already possible.

5.1. Role of NPS in cocaine reward and self-administration

The effect of NPS on the rewarding properties of cocaine was recently evaluated in our laboratory (Kallupi et al., 2010). Rats trained to self-administer cocaine under a fixed ratio were ICV injected with NPS. The cocaine self-administration was not affected by the NPS treatment, indicating that it does not play a role in cocaine reward. In the same study, we also tested the effect of the NPSR antagonist SHA 68 (Okamura et al., 2008); like NPS, this showed no effect on cocaine self-administration. Altogether these findings revealed that manipulation of NPSR did not modify the primary rewarding effects of cocaine. To some extent this is consistent with the CPP data (Fig. 1) indicating that NPS seems not to have a role in reward processing, as shown by the fact that it is devoid of motivational properties *per se* (Li et al., 2009).

5.2. Role of NPS in reinstatement of cocaine seeking and relapse

The first study linking NPS to cocaine relapse was published by Paneda et al. (2009). Using a drug priming paradigm, the authors found that ICV injection of NPS (0.45 nmol) reinstated extinguished lever pressing for cocaine in mice (Paneda et al., 2009). This effect appeared to be mediated by a downstream activation of the brain's CRF system, because it was prevented by administration of the CRF_1 receptor antagonist antalarmin. Moreover, using a genetic approach, the authors detected no differences in either the amount or the rate of cocaine self-administration between CRF_1 receptor knock out ($CRF_1(-/-)$) mice and their wild type (WT) littermates. However, when NPS was given ICV during extinction, it reinstated cocaine self-administration in the WT but not in the KO mice, further confirming a role for the CRF_1 system in NPS-induced relapse. NPS also failed to stimulate locomotor activity in CRF KO mice whereas, as expected, it increased total ambulatory activity in WT. Notably, the anxiolytic-like effect of NPS was preserved in CRF_1 KO mice, suggesting that this NPS property is independent of CRF_1 receptors (Paneda et al., 2009). The fact that the relapse promoting effects of NPS were inhibited by CRF_1 receptor blockade or deletion may suggest that this effect of the peptide depends upon activation of stress-related mechanisms; and, as is well known, stress, together with environmental conditioning factors, is one of the major determinants of relapse (see for review: Sarnyai et al., 2001; Shalev et al., 2002; Weiss et al., 2001). The pro-stress nature of NPS is supported by data indicating that intra-PaV NPS administration activates the HPA axis (Smith et al., 2006). However, in contrast to this hypothesis involving CRF-mediated stress mechanisms, there are other data indicating that anorexia following intra-PaV administration of NPS is not blocked by CRFR1 antagonism (Fedeli et al., 2009). Moreover, this hypothesis contrasts with the well documented anxiolytic-like nature of NPS (Fedeli et al., 2009; Jungling et al., 2008; Leonard et al., 2008; Rizzi et al., 2008; Vitale et al., 2008). An alternative possibility is that NPS shares some of the discriminative properties of cocaine; hence following extinction it may "prime" animals trained to cocaine self-administration. To thoroughly evaluate this hypothesis, drug discrimination studies will have to be carried out. A third possibility is that in the study by Paneda et al. (2009), NPS induced relapse was dependent upon the peptide's ability to increase goal oriented behaviors. In fact, in this study mice were trained to lever press for cocaine in the presence of a cue light contingently paired to lever pressing. The cue was maintained during the extinction phase, and then in the reinstatement test. NPS injection might have invigorated attention toward the cue (previously paired to cocaine) thus strengthening the animals' operant behaviors toward an incompletely extinguished signal, and therefore still predictive of a cocaine reward.

This view is, to some extent, supported by data recently generated in our laboratory where in a place preference extinction/reinstatement paradigm we found that cocaine conditioned rats resume preference following cocaine priming but not ICV NPS injection (Fig. 2). In the CPP/reinstatement model, the environment functions as a discriminative contextual stimulus, and animals are not required to emit instrumental goal-oriented responses toward a previously cocaine paired contingent stimulus.

Finally, it is noteworthy to mention that this alternative interpretation of the findings of Paneda et al. (2009) does not conflict with the involvement of CRF_1 mechanisms as suggested in their work. In fact, it is well known that extra-hypothalamic CRF not only controls stress and relapse mechanisms, but enhances attentional performances, facilitates attention shifting, and increases vigilance (Chen et al., 1992; Ohmura et al., 2009; Snyder et al., 2012).

Work conducted in our laboratory provides further strength for the role of the NPS system in the pathophysiology of cocaine craving and relapse. In a series of experiments using a conditioned reinstatement model of cocaine seeking, we found, in fact, that NPS potently reinstated relapse behavior following ICV or intra LH microinfusion. A significant but less pronounced effect was also observed after peptide administration into the PeF, while no activity was seen after injection into the DMH or the CeA (Kallupi et al., 2010). Of note, administration of the NPSR antagonist SHA 68 significantly reduced cue-induced reinstatement of cocaine seeking, revealing that NPS may play a physiopathological role in relapse (Kallupi et al., 2010).

Guided by our previous findings indicating that NPS facilitates conditioned reinstatement of alcohol-seeking through Hcrt-1/Ox-A mediated mechanisms, and by data showing that ICV NPS increases c-Fos expression in hypothalamic Hcrt-1/Ox-A cells (Cannella et al., 2009a; Kallupi et al., 2010; Niimi, 2006), we

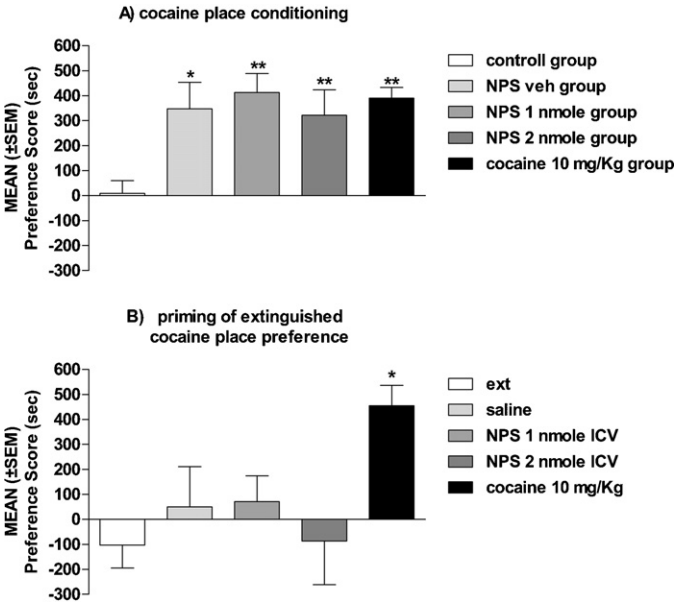

Fig. 2. Priming of extinguished cocaine conditioned place preference. (A) Cocaine place conditioning was measured in a two compartment apparatus using an unbiased schedule of conditioning. Rats ($n = 6–7$/group) underwent two conditioning sessions per day for three consecutive days. The NPS and cocaine groups received one daily injection of cocaine (10 mg/kg IP) in one compartment, and one of saline in the opposite, while the control group received two daily saline injections. Treatments were balanced across chambers and time. On the 4th day, drug free animals were allowed to explore the entire apparatus for 15 min; time spent in each chamber was recorded by an operator unaware of treatment conditions. One between factors ANOVA revealed an overall effect of conditioning [$F(4,26) = 4.36$; $p < 0.01$]; more detailed Newman–Keuls *post hoc* analysis revealed that all treated groups developed a conditioned place preference for the drug-paired compartment with respect to the control group. (B) Priming of extinguished cocaine place preference. Starting the day after the test for cocaine place conditioning, all groups except the control underwent one daily extinction session. Extinction sessions were similar to test sessions; extinction was considered achieved when the rat spent less than 70 s in the cocaine-paired side for three consecutive days. The day after the last extinction session, either NPS (1 or 2 nmol ICV) or cocaine (10 mg/kg IP) was injected to prime extinguished cocaine place preference. One between factors ANOVA revealed an overall effect of priming [$F(4,27) = 3.47$; $p < 0.05$]; Newman–Keuls *post hoc* analysis revealed that cocaine but not NPS treatment reinstated cocaine conditioned place preference. Preference scores are expressed as in Fig. 1, $^{*}p < 0.05$ and $^{**}p < 0.01$ with respect to control (A) or extinction (B).

investigated the effect of SB 334867 on NPS-induced facilitation of cue-induced cocaine relapse. Perfectly overlapping our previous results on alcohol, we found that blockade of Hcrt-1/Ox-A receptors completely prevented the effect of NPS on relapse.

Currently available data suggest, therefore, that the effect of NPS on cocaine relapse can be mediated by downstream activation of both the Hcrt-1/Ox-A and CRF1R receptor systems. The existence of a complex cross-talk between the Hcrt-1/Ox-A and the CRF systems has been well documented, but if, in mediating the effects of NPS, these two systems act in parallel or in sequence is not yet clear. At the neuroanatomical level, it has been shown that CRF-immunopositive cells make direct contact with Hcrt-1/Ox-A expressing neurons in the LH, and that numerous Hcrt-1/Ox-A neurons express CRF receptors. *In vitro* application of CRF depolarizes membrane potentials and increases firing rate in a subpopulation of Hcrt-1/Ox-A cells in the LH. Together these data suggest that CRF may act as an upstream modulator of the Hcrt-1/Ox-A system (Winsky-Sommerer et al., 2004). On the other hand, it has also been shown that, following ICV administration of Hcrt-1/Ox-A peptide, approximately 96% and 45% of CRF-containing neurons expressed Fos-like immunoreactivity in the PaV and the CeA, respectively (Sakamoto et al., 2004). Moreover, central injection of Hcrt-1/Ox-A peptide increased CRF and vasopressin expression in the parvocellular cells of the PaV, and stimulated ACTH release from the pituitary gland (Al-Barazanji et al., 2001). These findings point to the possibility that the CRF system is located downstream of the Hcrt-1/Ox-A network. With regard to the regulation of drug abuse dependent behaviors, the relationship between the CRF and the Hcrt-1/Ox-A systems appears even more complex. On the one hand, in fact, there are data indicating that these two peptidergic systems may work in sequence. For example, intracranial electrical self-stimulation experiments revealed that intra-VTA or ICV administration of Hcrt-1/Ox-A increased the self-stimulation threshold *via* subsequent activation of the CRF system (Hata et al., 2011). On the other hand, there are data indicating that the Hcrt-1/Ox-A and CRF systems may act in parallel through independent mechanisms. In fact, it has been shown that intra VTA infusion of Hcrt-1/Ox-A facilitated cocaine seeking, and caused release of VTA glutamate and dopamine that were blocked by the selective Hcrt-1/Ox-A antagonist SB-408124, but not by the CRF_1 antagonist α-helical CRF. Conversely, SB-408124 did not block CRF-dependent footshock-induced reinstatement, or glutamate or dopamine release (Wang et al., 2009). Moreover, ICV injection of Hcrt-1/Ox-A or footshock stress reinstated a previously extinguished nicotine-seeking behavior. The effects of Hcrt-1/Ox-A peptide were blocked by the Hcrt-1/Ox-A antagonist SB334867, but not by the CRF1R antagonist antalarmin. Conversely, stress-induced reinstatement of nicotine-seeking was prevented by antalarmin but not by SB334867 (Plaza-Zabala et al., 2010).

6. NPS and other substances of abuse

The only drug of abuse that, like NPS, possesses anxiolytic and tension relief properties associated with a stimulant-like profile is nicotine. The similarities between these two agents stimulated a relatively early study in which the effect of acute and chronic administration of nicotine on NPS and NPSR gene expression was investigated (Lage et al., 2007). Results showed that NPSR mRNA was increased in the brainstem after acute injection of nicotine. Chronic treatment with nicotine elicited an increase of NPS mRNA in the brainstem, and of NPSR mRNA both in the brainstem and the hypothalamus (Lage et al., 2007).

It has also been observed that the NPS system is likely to play a role in acute and chronic responses to caffeine (Lage et al., 2006). Acute treatment decreased NPS mRNA and increased NPSR mRNA in the brainstem, leaving expression of the NPSR precursor unaltered in the hypothalamus. Conversely, chronic treatment did not modify NPS and NPSR mRNA levels in the brainstem, while NPSR mRNA expression was increased in the hypothalamus (Lage et al., 2006).

The functional significance of these gene expression changes is unknown; however, the fact that exposure to psychoactive drugs affects the NPS system is intriguing. In this regard, it is noteworthy to mention that chronic exposure to alcohol increased NPSR gene expression; an event that was interpreted as part of a physiological compensatory response aimed at attenuating the heightened anxiety associated with alcohol withdrawal (Ruggeri et al., 2010).

7. NPS receptor antagonism as a treatment strategy for addiction

The availability of well-characterized pharmacological tools, in particular antagonists, is of fundamental importance for the study of the physiopharmacology of a biological system. Various peptidergic analogs of NPS (Camarda et al., 2009; Guerrini et al., 2009; Roth et al., 2006; Tancredi et al., 2007) endowed with agonistic or antagonistic properties have shown their usefulness in the characterization of the NPS system (Cifani et al., 2011; Fedeli et al., 2009; Kallupi et al., 2010). However, if on the one hand these

tools are useful in studying the neuropharmacology of the NPS system (*i.e.*, they are easily soluble in saline and can be centrally injected) on the other, they cannot be viewed as ideal tools to investigate the potential medical usefulness of NPSR antagonists.

Recently, non-peptidergic NPSR antagonists with favorable brain penetration profiles have been developed. The first to be published was 3-Oxo-1,1-diphenyl-tetrahydro-OxAzolo[3,4-*a*]pyrazine-7-carboxylic acid 4-fluoro-benzylamide, known as SHA 68 (Okamura et al., 2008). SHA 68 is a competitive antagonist for NPSR; its selectivity was established against 14 different GPCRs. Importantly, no agonistic or antagonistic activities were detected for vasopressin or oxytocin receptors, which, from the point of view of peptide sequence, are closely related to NPSR. In mice, brain penetration of these compounds is limited, but attainable at both low (5 mg/kg i.p.) and high (50 mg/kg i.p.) doses. At the lower dose, however, the brain concentration rapidly decayed, while it was more stable at the higher dose. Pharmacokinetic data were paralleled by *in vivo* studies, which demonstrated that the dose of 50 but not 5 mg/kg of SHA 68 was able to counteract the effect of NPS on locomotor activity. However, it has to be noted that SHA68 blocked only 50% of the hyperlocomotor effect of NPS (Okamura et al., 2008). A few weeks later, Zhang et al. (2008) published a series of SHA 68 analogs consisting of 7-substituted 1,1-diphenyl-tetrahydro-OxAzolo[3,4-*a*]pyrazine-3-ones with antagonistic properties for NPSR. Interestingly, most of their compounds were more potent against the Ile^{107} variant of NPSR (Zhang et al., 2008). As mentioned in the introduction the human receptor form hNPSR-A was found in two sub-variants given by a SNP at position 107 (Laitinen et al., 2004). Direct correlations between addiction and NPSR human polymorphisms have not yet been reported. However it is significant the observation that fear cause an activation of the dorsolateral prefrontal cortex (dlPFC) and lateral orbitofrontal cortex (lOFC) in individuals carrying the protective genotype A/A and a decreased activity in the anterior cingulate cortex (ACC) in individuals carrying the T risk allele (Domschke et al., 2011). This observation can be of interest also in the addiction field because dysfunction of the dlPFC and lOFC has been associated with drug dependence craving and relapse (Damasio, 1996; Lucantonio et al., 2012; Verdejo-Garcia and Bechara, 2009).

Based on current evidence it is not possible to draw definitive conclusions on the relationship between NPSR human polymorphism and addiction behaviors. However, it is tempting to anticipate that individuals carrying the A/A genotype could possibly show a higher resiliency to addiction while individuals carrying the T allele, could be more prone to impulsivity, and more susceptible to develop addictive-like behaviors. If this is the case, compounds that targets preferentially the NPSR Ile^{107} variant (Zhang et al., 2008) might represent a useful tool for individual-based therapies.

Two novel series of NPSR antagonists were also developed by Merck Laboratories. They identified NPSR-quinolinone-amide-1 (NPSR-QA1) as a potent NPSR antagonist, with sustained blood and cerebro-spinal-fluid (CSF) concentrations. The compound has a specific NPSR binding affinity, as it has not shown relevant off-target activities against a spectrum of 170 targets, yet it showed potent binding to rat NPSR (Melamed et al., 2010). The same company developed a second series of tricyclic-imidazole based antagonists, in which they identified the compound NPSR-PI1, which showed pharmacological properties similar to NPSR-QA1 (Kallupi et al., 2012; Trotter et al., 2010).

Despite the availability of various NPSR antagonists, to date only SHA68 has been subjected to behavioral screening (Kallupi et al., 2010; Okamura et al., 2008; Ruzza et al., 2010). It was shown that peripheral administration of SHA 68 was able to block the effect of NPS in various behavioral paradigms. Specifically, SHA68 reduced hyperlocomotion induced by NPS; it blunted the increase in time spent in the central area of an open-field but not the number of entries; it reduced the anxiolytic-like effect of NPS in the elevated plus maze and the defensive burying tests; and slightly counter-acted the anorectic effect of NPS on palatable food intake. Thus, SHA 68 demonstrated its antagonist properties *in vivo* in a wide set of tests without showing behavioral effects *per se* at the doses tested (Ruzza et al., 2010). In the only study in which NPS antagonists were tested on behavior related to addiction, it was shown that SHA 68 did not affect cocaine self-administration but markedly reduced cue-induced reinstatement of cocaine seeking (Kallupi et al., 2010).

Stress and anxiety are two major factors implicated in the maintenance and reinstatement of addictive behavior (Koob, 2009). NPS was shown to possess anxiolytic-like properties, but at the same time acts as a pro-arousal and a pro-stress mediator (Smith et al., 2006; Xu et al., 2004). Hence, NPSR antagonists might have anti-stress properties but also anxiogenic-like potential. Noteworthy, studies on SHA 68 revealed that this compound (and possibly other NPSR antagonists), was able to block reinstatement of cocaine seeking at doses that did not show anxiogenic or hypoarousal effects *per se* (Kallupi et al., 2010; Okamura et al., 2008; Ruzza et al., 2010). Given the activating role of NPS on HPA axis function (Smith et al., 2006), and considering that the NPS system and the HPA axis are both activated in post-dependent rats (Rasmussen et al., 2000; Zorrilla et al., 2001), it is reasonable to predict that NPSR antagonist could show benefit in the treatment of withdrawal to drugs.

8. Conclusions

The data reviewed here demonstrate that 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., 2009a; Kallupi et al., 2010; Paneda et al., 2009). As previously discussed in drug addicts the relapse risk is particularly high during withdrawal, a condition often associated with a high anxiety. One possibility is that, in the presence of an anxious state, the NPS system is recruited for compensatory mechanisms. But because NPS also stimulates arousal its activation may ultimately contribute to exacerbate drug seeking and relapse. Hence, overactivity of the NPS system may be part of those neuroadaptive changes occurring in addiction.

Altogether these data indicate that the NPS/NPSR system may have a role in some aspects of drug addiction. Particularly attractive is the possibility that NPSR antagonists may be useful in the management of drug craving and relapse.

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