


# Neurotensin: A role in substance use disorder?

Luca Ferraro<sup>1,2</sup>, Laura Tiozzo Fasiolo<sup>1</sup>, Sarah Beggiato<sup>3</sup>,  
Andrea C Borelli<sup>3</sup>, Lucyna Pomierny-Chamiolo<sup>4</sup>,  
Malgorzata Frankowska<sup>5</sup>, Tiziana Antonelli<sup>3</sup>, Maria C Tomasini<sup>1</sup>,  
Kjell Fuxe<sup>3</sup> and Malgorzata Filip<sup>5</sup>

*Journal of Psychopharmacology*  
2016, Vol. 30(2) 112–127  
© The Author(s) 2016  
Reprints and permissions:  
sagepub.co.uk/journalsPermissions.nav  
DOI: 10.1177/0269881115622240  
jop.sagepub.com  


## Abstract

Neurotensin is a tridecapeptide originally identified in extracts of bovine hypothalamus. This peptide has a close anatomical and functional relationship with the mesocorticolimbic and nigrostriatal dopamine system. Neural circuits containing neurotensin were originally proposed to play a role in the mechanism of action of antipsychotic agents. Additionally, neurotensin-containing pathways were demonstrated to mediate some of the rewarding and/or sensitizing properties of drugs of abuse.

This review attempts to contribute to the understanding of the role of neurotensin and its receptors in drug abuse. In particular, we will summarize the potential relevance of neurotensin, its related compounds and neurotensin receptors in substance use disorders, with a focus on the preclinical research.

## Keywords

Agonist, antagonist, behavioral sensitization, conditioned place preference, drug abuse, drug self-administration, hyperlocomotion, neurotensin, neurotensin receptor, preclinical research, review

## Introduction

Neurotensin (NT), a tridecapeptide originally identified in extracts of bovine hypothalamus by Carraway and Leeman (1973), is widely distributed in the central nervous system (CNS) and in the periphery, including the gut, pancreas and adrenal glands (Carraway and Leeman, 1976; Kitabgi et al., 1976). The biological actions of NT are initiated by binding to three different receptor subtypes: neurotensin receptor subtype 1 (NTS1), NTS2 and NTS3 (Geisler et al., 2006; Mazella and Vincent, 2006). NTS1 and NTS2 receptors belong to the family of G-protein-coupled receptors (Pelaprat, 2006). These NT receptor subtypes differ in their affinity for NT and their sensitivity to levocabastine, an antihistaminic compound.

The NTS1 receptor is levocabastine-insensitive, with a high affinity for NT; whereas the NTS2 receptor is levocabastine-sensitive, with a low affinity for NT (St-Gelais et al., 2006; Vincent et al., 1999). The NTS1 receptor is the best characterized NT receptor subtype. It is functionally coupled to the phospholipase C and the inositol phosphate signaling cascade, but its activation has been also associated with cyclic guanosine monophosphate, cyclic adenosine monophosphate and arachidonic acid production, along with mitogen-activated protein (MAP) kinase phosphorylation. The transduction mechanisms of the NTS2 receptor remain a matter of controversy (Mazella and Vincent, 2006). NT3 is structurally unrelated to these receptors, belongs to the family of sorting receptors and modulates NT intracellular signaling processes (Mazella and Vincent, 2006).

By interacting with its receptors, NT is known to exert several effects in mammals, including analgesia, hypothermia, neuroendocrine control of thyroid hormones, regulation of blood pressure and

body weight homeostasis. NT is also deeply involved in immunity and inflammation; but its true role in these events still remains to be elucidated (Katsanos et al., 2008), as well as its relevance in cancer development and progression (Myers et al., 2009). When injected systemically, NT decreases blood pressure, gastric motility, gastric acid secretion and induces hyperglycemia.

The differential regional distribution of NT in the brain, the presence of NT receptors in different brain areas (such as cortical, striatal and limbic regions), the sodium- and calcium-dependent NT release and the involvement of NT in several electrophysiological and behavioral responses (Carraway and Leeman, 1973; Kitabgi et al., 1989; Saint-Gelais et al., 2006; Tanganelli et al., 1994; Vincent et al., 1999), suggest that this peptide acts as a neurotransmitter or neuromodulator in the mammalian CNS. In this context, the effects of NT include the well-documented interaction of the peptide with dopaminergic

<sup>1</sup>Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy

<sup>2</sup>Laboratory for Technologies of Advanced Therapies (LTAT), University of Ferrara, Ferrara, Italy

<sup>3</sup>Department of Medical Sciences, University of Ferrara, Ferrara, Italy

<sup>4</sup>Department of Toxicology, Medical College, Jagiellonian University, Kraków, Poland

<sup>5</sup>Laboratory of Drug Addiction Pharmacology, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland

## Corresponding author:

Luca Ferraro, Department of Life Sciences and Biotechnology, University of Ferrara, Via Fossato di Mortara 17–19, 44121 Ferrara, Italy.  
Email: [frr@unife.it](mailto:frr@unife.it)

(DAergic) systems (Geisler et al., 2006; Kitabgi et al., 1989; Rostène et al., 1992; Von Euler and Fuxe, 1987). This is mainly due to the 'antagonistic' action of the activated NTS1 on the dopamine (DA) D2 receptor recognition and signaling, via an intra-membrane NTS1/DA D2 receptor-receptor interaction (Antonelli et al., 2007b; Fuxe et al., 1992; Von Euler, 1991; Von Euler and Fuxe, 1987; Von Euler et al., 1991). In particular, pioneer binding studies demonstrated that, by means of an intra-membrane NTS1/DA D2 receptor-receptor interaction, NT reduced the affinity of the DA D2 receptor agonist (Agnati et al., 1983). More recently, it has been reported that the dynamic changes in DA D2 receptor signaling produced by NT receptor agonists may involve antagonistic allosteric receptor-receptor interactions in the NTS1/D2 receptor heteromers at the plasma membrane level (cAMP response element-binding protein (CREB) pathway) and synergistic interactions in PKC activation at the cytoplasmic level (mitogen-activated protein kinases (MAPK) pathway). The literature on NTS1/DA D2 receptor interactions and the possible relevance of this heterodimer in the CNS were recently reviewed (Ferraro et al., 2014; Tanganelli et al., 2012).

NT also appears to modulate the activity of other neurotransmitter circuits that are innervated by the DA system. Neural circuits containing NT were originally proposed to play a role in the mechanism of action of antipsychotic agents (Boules et al., 2007; Boules et al., 2014; Kinkead and Nemeroff, 2004; Nemeroff, 1980). In particular, the interaction between NT and DA has been implicated in the pathogenesis and treatment of schizophrenia (Boules et al., 2014; Caceda et al., 2006; LaCrosse and Olive, 2013; Nemeroff et al., 1983; Tanganelli et al., 2012). In fact, it has been reported that NT and the NT agonists possess neuroleptic-like properties in DA-mediated animal models of psychosis, such as amphetamine-induced locomotor activity, apomorphine-induced climbing and drug-induced disruption of pre-pulse inhibition (PPI) (Boules et al., 2001; Kalivas et al., 1983; 1984; Nemeroff et al., 1983; Shilling et al., 2003).

Besides DA systems, growing evidence suggests that NT may also play an important role in the modulation of aminoacidergic transmission in the basal ganglia and cerebral cortex (Antonelli et al., 2007a; Ferraro et al., 2008, 2009). In particular, NT amplifies glutamate transmission and enhances glutamate-induced excitotoxicity. The hypothesis was therefore introduced that NT may be involved in ischemic brain damage and neurodegenerative disorders (Antonelli et al., 2007b, 2008; Ferraro et al., 2009).

Several reviews on the relevance of NT in schizophrenia and neurodegenerative disorders have been published (Antonelli 2007b; Boules et al., 2014; Ferraro et al., 2008; 2009; Kinkead and Nemeroff, 2004; LaCrosse and Olive, 2013; Saint-Gelais et al., 2006; Tanganelli et al., 2012), underlining the evidence that the NT receptor agonists or antagonists may represent novel antipsychotic and neuroprotective drugs, respectively. In contrast, the important role played by central NT receptor mechanisms in drug addiction has only partially been reviewed (Boules et al., 2014; Ferraro et al., 2007), which is also true regarding their relevance for drug abuse treatment. This is probably due to the complex and partially controversial results emerging from the studies in this field. They demonstrate that the effect of NT depends on many parameters, such as: the dose used, the injection procedure and the NT receptor reached, as well as the experimental model or species used. Nevertheless, the possible implication of NT in drug abuse (Saint-Gelais et al., 2006) is supported by substantial experimental

evidence and the similarities between certain NT- and psychostimulant drug-induced effects.

This review attempts to contribute to the understanding of the role of NT and its receptors in drug abuse, by also discussing the possible relevance of NTS1/DA D2 receptor heterocomplexes. To this purpose, the potential relevance of NT, its related compounds and NT receptors in substance use disorders will be summarized, focusing on preclinical research.

## Neurotensin in the central nervous system

### *Biochemistry and localization*

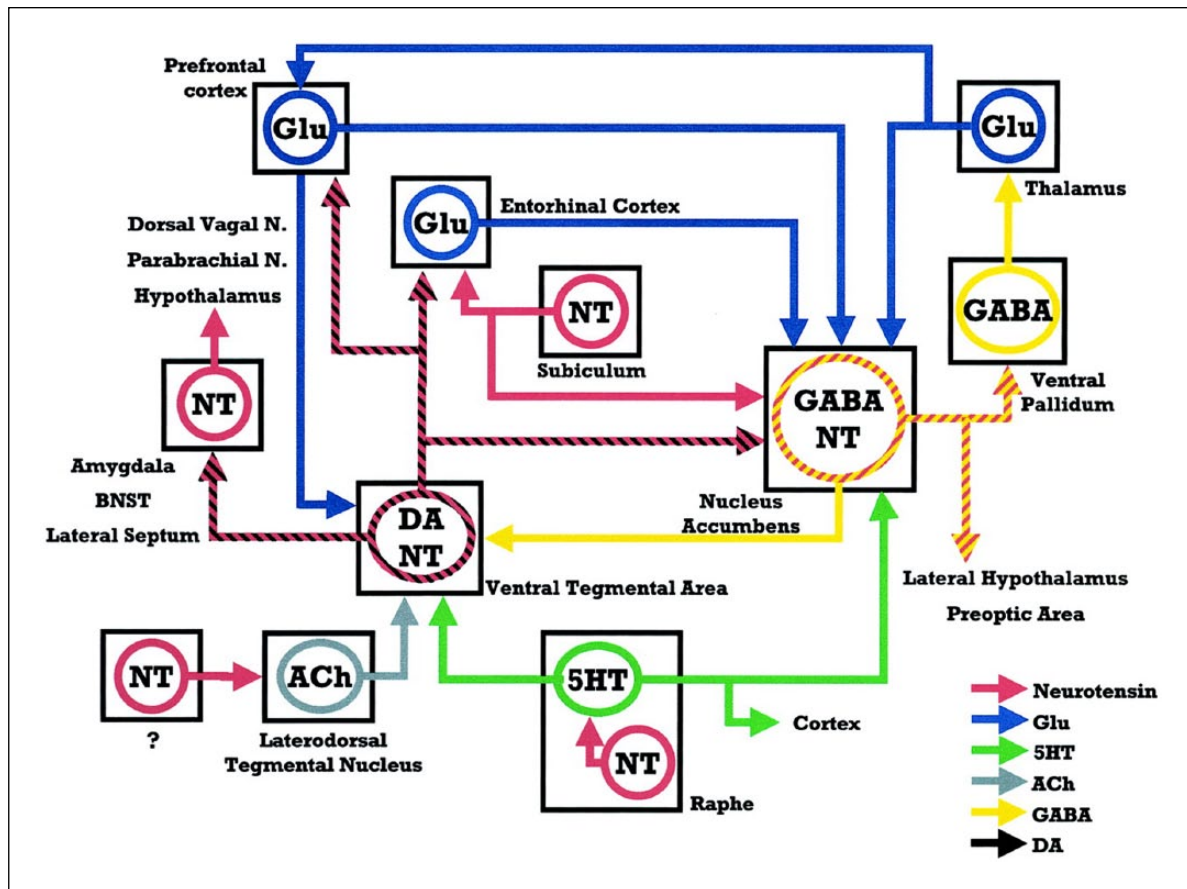
Within the CNS, NT is synthesized, stored at specific synapses and synaptic varicosities and, under appropriate conditions, it may be released or co-released with classical neurotransmitters. NT and its structurally related hexapeptide neuromedin N (NN) are products of the same larger precursor, whose cDNA was cloned from bovine brain in 1987 (Dobner et al., 1987; Minamino et al., 1988). The precursor molecule, a highly conserved polypeptide of 169 or 170 amino acids in length, contains one copy each of NT and NN near the C-terminus; and it undergoes a differential tissue-specific cleavage at its four dibasic sites, by pro-protein convertases (PCs). Therefore, pro-NT/NN may be processed to generate different sets of peptides. Four biologically active products of pro-NT/NN processing have been described: NT, NN, large NT and large NN (Kitabgi, 2010). In the brain, pro-NT/NN processing mainly depends on PC2 activity and leads to high amounts of NT and NN, and small quantities of large NT and large NN (Kitabgi, 2010). Using radioimmunoassay techniques, it was demonstrated that the regional distribution of NT and NN in brain tissues is, generally, the same (Kitabgi et al., 1992); however, marked differences in the ratio of NT over NN have been observed in different brain areas, with NT being generally more abundant in the DAergic regions such as the substantia nigra, pars compacta and ventral tegmental area (VTA).

Once processed as an active peptide in neurons, NT is stored in dense core vesicles and released in a calcium-dependent manner (Iversen et al., 1978). The physiological inactivation of NT is operated by endopeptidases (EPs) belonging to the family of metallo-peptidases, which act on primary cleavage sites in the peptide sequence: Arg8-Arg9, Pro10-Tyr11 and Tyr11-Ile12 bonds. Three EPs are responsible for NT degradation: EP 24.11, EP 24.15 and in particular, EP 24.16, which is ubiquitously expressed (Kitabgi, 2006). Other exo- and endopeptidases further degrade the breakdown products generated by these metallopeptidases. Another mechanism that produces an inactivation of NT transmission is the process of NT internalization (Mazella and Vincent, 2006).

### *NT pathways*

Several NT-containing neuronal circuits have been described in the rat. Among others (Saint-Gelais et al., 2006), these include the neurons projecting from:

- The amygdala to the striae terminalis, the substantia nigra, pars compacta, the substantia nigra, pars reticulata and to the ventromedial nucleus of the hypothalamus;



**Figure 1.** Schematic representation of direct and indirect associations between NT projections and the mesocorticolimbic DA system. The cross-hatched lines represent projections colocalizing either the NT and DA (black and red), or NT and GABA (yellow and red).

Source: Reproduced from Binder EB, Kinkead B, Owens MJ, et al. (2001) Neurotensin and dopamine interactions. *Pharmacol Rev* 53: 453–486 with permission from Baltimore, Williams & Wilkins.

ACh: acetylcholine; BNST: bed nucleus of the stria terminalis; DA: dopamine; GABA:  $\gamma$ -aminobutyric acid; Glu: glutamate; 5HT: 5-hydroxytryptamine; NT: neurotensin.

- The hippocampus through the cingulate cortex to the frontal cortex;
- The hypothalamus, the ventromedial ventral pallidum, the dorsal raphe nucleus and the diagonal band of Broca to the VTA; and
- The cells in the VTA to the nucleus accumbens (NAC), the amygdala, and especially the prefrontal cortex (PFC), from the striatum to the substantia nigra, pars reticulata.

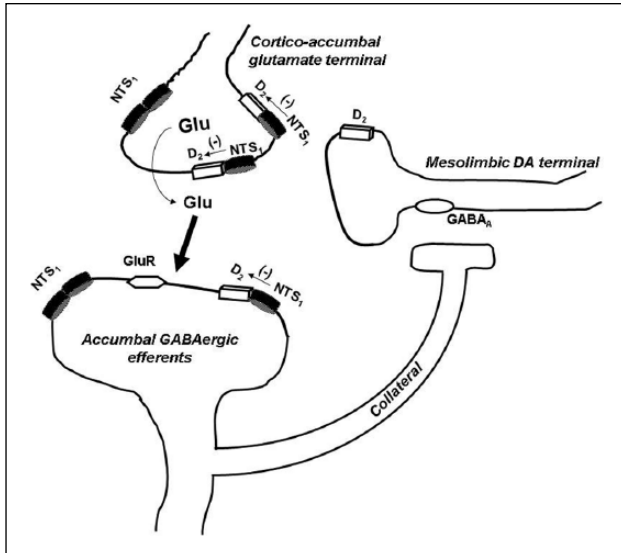
High levels of the peptide are present in the hypothalamus, amygdala, bed nucleus of the striae terminalis, lateral septum, NAC, caudate-putamen and VTA (Geisler et al., 2006; Tyler-McMahon et al., 2000). Such a distribution of the peptide generally matches the distribution of the NT receptors in the brain (Geisler et al., 2006; Vincent et al., 1999).

#### *NT and DA signaling: Focus on mesolimbic transmission*

Berger et al. (1992) demonstrated that in the rat, but not in primates, NT is in part co-localized with DA in mesocortical

neurons, but not in nigrostriatal and mesolimbic DA neurons, thus suggesting that NT might play a special role in the regulation of mesocortical DA transmission (Kalivas and Miller, 1984; Von Euler et al., 1990); however, the class of mesocortical DA projections that are not co-localized with NT in rat are particularly developed in human, as are the NT projections to the limbic system. These findings strengthen the role of NT corticolimbic innervations in primate brain. The mixed NT/DA neurons project to the PFC, entorhinal cortex, NAC, basolateral nucleus of the amygdala and lateral septum (Figure 1). NT/DA projections mostly overlap mesocorticolimbic DA projections with the exception of the central nucleus of the amygdala and the NAC core, where there are no mixed projections (Binder et al., 2001). NT fibers innervate the VTA and possibly originate from the rostral lateral septum, the pre-optic area and the lateral hypothalamus (Binder et al., 2001).

The activation of somatodendritic NTS1 receptors increases the firing rate of mesolimbic and mesocortical DAergic neurons (Werkman et al., 2000), most likely by increasing intracellular  $Ca^{2+}$  and reducing  $K^+$  conductances. NT administered to the VTA acutely excites DA neurons (Shi and Bunney, 1990; Werkman et al., 2000) and induces increased turnover and extracellular



**Figure 2.** Schematic representation of the main location of the  $NTS_1/D_2$  heteromers mediating the  $NTS_1/D_2$  receptor-receptor interactions in the control of the ventral striato-pallidal GABA pathway from the nucleus accumbens. In the nucleus accumbens, there exists a dominance of the post-junctional antagonistic  $NTS_1/D_2$  receptor-receptor interactions on the cortico-accumbens glutamate terminals over the weak antagonistic  $NTS_1/D_2$  receptor-receptor interaction on the accumbens DA terminals (not shown). The intra-accumbens perfusion of NT antagonizes the inhibitory dopamine  $D_2$  tone on glutamatergic terminals via a  $NTS_1/D_2$  receptor-receptor interaction, thus inducing an enhancement of glutamate outflow. Such an increase can then activate the inhibitory GABAergic signaling that could be responsible, via a collateral, for the significant reduction of DA release observed in the nucleus accumbens (Tanganelli et al., 1994); however, a direct involvement of  $NTS_1$  located on GABAergic neurons (Binder et al., 2001) cannot be excluded. The two  $D_2/NTS_1$  heteromers on the glutamate terminals are to show that this is their major location, since only few are found on the striato-pallidal GABA neurons.  $NTS_1$  alone without  $D_2$  could be a monomer or a homomer, which is true also for  $D_2$  when not present in a heteromer. Source: Adapted from Ferraro L, Tomasini MC, Fuxe K, et al. (2007) Mesolimbic dopamine and cortico-accumbens glutamate afferents as major targets for the regulation of the ventral striato-pallidal GABA pathways by neurotensin peptides. *Brain Res Rev* 55: 144–154 with permission from Elsevier.  $D_2$ : dopamine  $D_2$  receptor; DA: dopamine;  $GABA_A$ : type-A  $\gamma$ -aminobutyric acid receptors; Glu: glutamate; GluR: glutamate receptor; NT: neurotensin;  $NTS_1$ : neurotensin receptor subtype 1.

concentration of DA in the NAC (Kalivas and Duffy, 1990; Steinberg et al., 1995). Concerning the NAC, NT receptors are co-localized with postjunctional DA receptors on glutamate terminals and on soma-dendrites of striato-pallidal GABA neurons, although this matter is still controversial and inconsistent (Ferraro et al., 2007). Graphic representation can be seen in Figure 2.

Interestingly, NT was reported to either increase or decrease NAC DA transmission, depending on the dose (Boules et al., 2014; Ferraro et al., 2007). It was suggested that intra-NAC NT application preferentially modulates prejunctional DAergic transmission, mainly via indirect mechanisms involving other neuronal systems, rather than through a direct activation of the few  $NTS_1$  receptors located on the NAC DAergic terminals. In particular, it seems likely that the peptide, by activating  $NTS_1$

receptors mainly located on NAC glutamate terminals, induces an enhancement of extracellular DA levels. One of the possible mechanisms underlying this effect may be an indirect inhibitory action of the peptide on glutamate terminal DA  $D_2$  receptors (Agnati et al., 1983; Fuxe et al., 1992). The extracellular increase in NAC glutamate levels can then activate the inhibitory GABAergic signaling of dendrites and collaterals of the ventral striato-pallidal GABA pathway, which could be responsible for the significant reduction of extracellular DA levels observed in the NAC, as seen in Figure 2 (Tanganelli et al., 1994). This mechanism could be involved in the neuroleptic-like action of NT. In contrast to the NAC, NT increases DAergic signaling in the dorsal striatum, mainly via the activation of a relatively high density of  $NTS_1$  receptors located on striatal DA terminals involving inhibition of the DA  $D_2$  autoreceptors (Li et al., 1995; Tanganelli et al., 1994).

## Neurotensin and substance use disorders

Substance use disorder refers to alcohol/drug abuse or alcohol/drug dependence (American Psychiatric Association, 2013). Impairments due to drugs of abuse start in the brain reward circuits, including the mesolimbic DA system (as all the addictive substances enhance DAergic transmission); while long-term drug intake leads to dysfunctions of the brain regions involved in learning and memory, habit-forming learning and inhibitory control, being under control of the glutamatergic or GABAergic neurotransmissions (Tzschenke and Schmidt, 2003). Wide NT distribution in the brain and its localization to DAergic rich areas, as well as its direct or indirect modulation of glutamatergic and GABAergic transmission, speak for the interaction of NT with drugs of abuse belonging to different chemical classes. Below, we describe and discuss the preclinical behavioral evidence (based on acute and repeated drug treatments) on the role of NT in chemical addictions.

## Neurotensin and drug of abuse-evoked hyperlocomotion

Locomotor hyperactivity mainly depends on the stimulation of the meso-NAC DAergic neurons, projecting from the VTA to NAC, which constitutes the so-called ‘reward’ pathway (Filip and Siwanowicz, 2001; Hedou et al., 1999). Because locomotor hyperactivity was proposed as an index of the stimulatory effects of drugs of abuse (Phillips and Shen, 1996; Wise and Bozarth, 1987) that use the same neuroanatomical pathway to enhance their rewarding properties, the effects of NT on spontaneous and drug-induced locomotion will be summarized in the present section.

Several studies have focused on the investigation of the behavioral effects induced by acute NT administration, leading to somewhat controversial conclusions (Table 1). Thus, pioneering behavioral studies demonstrate that microinjection of NT into the NAC leads to a reduction of locomotion (Kalivas et al., 1984; Meisenberg and Simmons, 1985; Tanganelli et al., 2012). Conversely, the non-peptide  $NTS_1$  receptor antagonist SR48692 reduces both haloperidol-induced hypolocomotion and haloperidol reversal of amphetamine-induced hyperlocomotion. These findings point to the inhibitory role of NT on  $NTS_1$  receptors in locomotor activity.

**Table 1.** Neurotensin and drug of abuse-evoked hyperlocomotion.

Drug (dose range and administration route)	Drug of abuse (dose range and administration route)	Species (gender)	Hyper-locomotion change	Reference
<b>Neurotensin</b>				
NT (30 µg; i.c.)	Amph (1–3 mg/kg; i.p.)	SD rats (male)	↓	Skoog et al., 1986
NT (30 µg; i.c.v.)	Amph (3 mg/kg; i.p.)	OF1 mice (male)	↓	Sarhan et al., 1997
NT (2.5 µg; intra-VTA injection)	Amph (0.5 mg/kg; i.p.)	SD rats (male)	0	Elliott and Nemeroff, 1986
NT (4.2, 6.7 µg; intra-NAC injection)	Cocaine (15 mg/kg; i.p.)	Wistar rats (male)	↓	Robledo et al., 1993
NT (4.2, 16.7 µg; intra-NAC injection)	Amph (0.75 mg/kg; i.p.)	Wistar rats (male)	↓	Robledo et al., 1993
<b>Neurotensin analogues and agonists</b>				
NT1 (0.3 mg/kg; i.p.)	Amph (3 mg/kg; i.p.)	OF1 mice (male)	↓	Sarhan et al., 1997
NT69L (0.10 µmol/kg; s.c.)	Amph (0.5 mg/kg; s.c.)	Wistar rats (male)	↓	Hertel et al., 2001
NT69L (1 mg/kg; i.p.)	Amph (0.75–5 mg/kg; i.p.)	SD rats (male)	↓	Boules et al., 2001
NT69L (1 mg/kg; for 1, 3 or 5 days; i.p.)	Amph (5 mg/kg; i.p.)	SD rats (male)	↓	Boules et al., 2003
NT69L (1 mg/kg; i.p.)	Cocaine (4–40 mg/kg; i.p.)	SD rats (male)	↓	Boules et al., 2001
NT69L (1 mg/kg; for 3 or 5 days; i.p.)	Cocaine (40 mg/kg; i.p.)	SD rats (male)	↓	Boules et al., 2003
PD149163 (1 mg/kg; for 8 days; s.c.)	Amph (0.5 mg/kg; s.c.)	SD rats (male)	↓	Feifel et al., 2008
<b>Neurotensin antagonists</b>				
SR 48692 (0.1–1 mg/kg; i.p.)	Amph (2.5 mg/kg; i.p.)	CD1 mice (male)	0	Casti et al., 2004
SR 48692 (1 mg/kg; i.p.)	Cocaine (15 mg/kg; i.p.)	SD rats (male)	0	Betancur et al., 1998
SR 48692 (1 mg/kg; for 5 days; i.p.)	Cocaine (15 mg/kg; i.p.)	SD rats (male)	↓	Betancur et al., 1998
SR 142948A (0.03–0.1 mg/kg; i.p.)	Amph (2.5 mg/kg; i.p.)	CD1 mice (male)	0	Casti et al., 2004
SR 142948A (100 µg/kg; i.p.)	Amph (2 mg/kg; i.p.)	SD rats (male)	0	Caceda et al., 2012
SR 142948A (1 mg/kg; i.p.)	MDMA (9 mg/kg; i.p.)	CD1 mice (male)	↓	Marie Claire et al., 2008

↓ (arrow down): decrease; Amph: amphetamine; CD1: cluster of differentiation 1; i.c.: intracisternal; i.c.v.: intracerebroventricular; i.p.: intraperitoneal; MDMA: 3,4-methylenedioxymethamphetamine; NAC: nucleus accumbens; NT: neurotensin; 0: no change; OF1: Oncins France 1; s.c.: sub-cutaneous; SD: Sprague Dawley; VTA: ventral tegmental area.

More recently, the involvement of NT signaling in the psychostimulant properties of drugs of abuse was extensively explored (Table 1). The intracerebroventricular (i.c.v.) or intracisternal (i.c.) administration of NT attenuated amphetamine-induced hyperlocomotion and reward behaviors induced by cocaine (Sarhan et al., 1997). Furthermore, intra-NAC injection of the peptide significantly decreased cocaine-induced and, at a higher dose, amphetamine-elicited hyperlocomotion. This suggested the ability of the peptide to modulate the psychostimulant-induced locomotor activation through an interaction with the mesolimbic DA system (Robledo et al., 1993; Sarhan et al., 1997). In line with the above observations, the acute systemic administration of the brain-penetrating NT analogue NT69L (N-methyl-L-Arg, L-Lys, L-Pro, L-*neo*-Trp, L-*tert*-Leu, L-Leu) reduced spontaneous locomotor activity, as well as the hyperlocomotion caused by both amphetamine and cocaine; however, the inhibitory effect of NT69L was lost, following repeated drug administration, due to a desensitization of NT receptors (Boules et al., 2001, 2003; Hertel et al., 2001).

Other investigators demonstrated that the i.p. injections of NTS1, a brain-penetrable NT-related peptide, decreases the amphetamine-stimulated hyperlocomotion (Sarhan et al., 1997); whereas the systemic administration of PD149163, a selective and potent NTS1 receptor agonist, counteracted the locomotor effects of amphetamine in both acute and subchronic regimens (Feifel et al., 2008). In contrast to the above-cited literature, other studies suggest that some behavioral effects of NT are similar to those induced by peripheral administration of psychomotor stimulants (Boules et al., 2013; Richelson et al., 2003); however, such excitatory locomotor effects are associated with results

obtained with intra-VTA NT microinjection, and are abolished by both i.c.v. injection of haloperidol and the destruction of the meso-NAC DAergic pathway (Kalivas and Taylor, 1985; Kalivas et al., 1983). Thus, it was proposed that NT stimulates locomotor activity through activation of DA neurons, when it is injected at the level of the VTA DAergic cell bodies (Kalivas and Duffy, 1990; Kalivas et al., 1983). Instead, the peptide inhibits locomotion through a post-synaptic modulation of neuronal systems regulated by NAC DA terminals (Kalivas et al., 1984). The latter effects involve an inhibitory action exerted by NTS1 receptor activation on D2 receptor function, through a post-junctional NT-D2 receptor-receptor interaction (Fuxe et al., 1992); while in the VTA, the existence of NTS1-D2 autoreceptor complexes on DA nerve cells has been proposed (Borroto-Escuela et al., 2013). NTS1 receptors inhibit DA D2 autoreceptor signaling in such heterocomplexes; and thereby, increase the firing in the DA neurons (Ferraro et al., 2014).

Other studies investigated the effects of NT receptor antagonists, such as SR48692 and SR142948A, on locomotor hyperactivity after acute treatment with drugs of abuse. SR48692 is widely used as a NTS1 receptor antagonist, as it displays higher affinity for NTS1 than for NTS2 receptors (Boules et al., 2006). Some authors also report that SR48692 acts as an agonist at NTS2 receptors (Botto et al., 1997; Richard et al., 2001; Vita et al., 1998). SR142948A is considered a non-selective NT receptor antagonist (Bose et al., 2015); however, Vita et al. (1998) provided evidence that SR142948A acts as an agonist, not an antagonist, at NTS2 receptors. Neither SR48692 nor SR142948A had any effect on mouse spontaneous motor activity or amphetamine- and cocaine-elicited hyperlocomotion (Betancur et al., 1998; Caceda et al.,

2012; Casti et al., 2004). This suggests that endogenous NT does not modulate spontaneous and drug-induced locomotion. On the other hand, repeated systemic injection of SR48692 reduced the behavioral response to acute cocaine (Betancur et al., 1998); and the acute systemic injection of SR142948A blocked the hyperlocomotion elicited by 3,4-methylenedioxymethamphetamine (MDMA), providing evidence for the possible involvement of the neuropeptide in the behavioral activation induced by some psychostimulants through the release of NT at the level of the DA cell bodies in the ventral midbrain.

In conclusion, the above results led us to hypothesize that NT analogs or selective NTS1 receptor agonists that cross the brain blood barrier after their systemic administration might be useful, clinically in modulating hyperactivity and certain behavioral responses to drugs of abuse (Boules et al., 2006; Vадnie et al., 2014); however, some of these effects seem to be reachable also by using NT receptor antagonists (Betancur et al., 1998) and other studies argue against the involvement of endogenous NT signaling in the hyperlocomotion elicited by psychostimulant drugs (Hall et al., 2012). It seems possible that this could be due to the use of unselective NT receptor ligands, the different neuronal localization of NT receptors in several brain areas and the heteromeric receptor complexes, in which NT receptor protomers participate, that under different addiction phases may evoke an opposite action; however, further and more extensive investigations are necessary to possibly clarify the role of the peptide in drug of abuse-evoked locomotor behaviors.

## Neurotensin and drug of abuse-evoked sensitization

Repeated, intermittent administrations of drugs of abuse induce a strong enhancement in locomotor stimulation that may last for a long time. This phenomenon, also called locomotor sensitization or behavioral sensitization, has been suggested to predict the addictive property of a drug combined with forms of neuronal adaptations linked to an enhancement of the reinforcing and motivational aspects of drugs of abuse. Two separate temporal domains of drug-induced sensitization within neuronal networks have been identified and termed initiation and expression. The initiation of behavioral sensitization to psychostimulants is operationally defined as the transient sequence of cellular and molecular events precipitated by psychostimulant administration, which ultimately leads to enduring changes in neural function.

Expression is defined as the enduring neural alterations arising from the initiation process that directly mediate the sensitized behavioral response (Robinson and Berridge, 1993; Stekete and Kalivas, 2011). Initiation and expression of locomotor sensitization are reported to have distinct neurochemical mechanisms, and different brain structures are involved in the two temporal phases: initiation of behavioral sensitization to psychostimulants is mostly related to DAergic and glutamatergic VTA transmission, while the neuronal changes associated with sensitization expression are mainly localized among the interconnection between VTA, NAC, PFC and amygdala (the so-called 'motive circuit'), which leads to a drug-induced increase in DA and glutamate release in the NAC. Moreover, the neuronal adaptations involved in the expression of behavioral sensitization to different drugs of abuse are shown to be distinct in the motive circuit (i.e. cocaine-elicited sensitization appears to involve more descending corticofugal excitatory

afferents than amphetamine) and result in an altered balance between the interconnections of several neurotransmitter systems (Pierce and Kalivas, 1997). Numerous studies have evaluated the possible involvement of NT in psychostimulant sensitization, giving mixed results (Tables 2(a), 2(b) and 2(c)).

## Development of sensitization

The first evidence indicated that chronic pre-exposure to the NTS1 receptor antagonist SR48692 (without cocaine injections) before the development of cocaine sensitization reduced the latter phenomenon, whereas the same drug given daily before cocaine injections had no effect on cocaine sensitization (Betancur et al., 1998; Horger et al., 1994). On the other hand, SR48692 dose-dependently attenuated and/or prevented the development of sensitization to amphetamine (Panayi et al., 2002; Rompré and Perron, 2000), pointing to the role of NAC NTS1 receptors in attenuating the sensitizing locomotor actions of such drugs of abuse. Interestingly, the other NTS1/2 receptor antagonist, SR142948A, acutely administered with amphetamine, failed to effect the acquisition of drug sensitization (Panayi et al., 2005). These discrepancies have been proposed to be due to the different drug administration routes, the drug treatment schedule or to other methodological variables (Panayi et al., 2005; Rompré and Perron, 2000). Also, differences in their ability to counteract DA D2 postjunctional versus autoreceptor signaling in heterocomplexes should be considered, as well as the NT receptor antagonist selectivity toward NTS1 or NTS1/2 receptors.

Based on these data, it can be speculated that NT might act on some neural mechanisms that support the neuroadaptations underlying psychostimulant drug sensitization. To support this hypothesis, it was reported that NT is released in the VTA after amphetamine injection; and that it contributes, although not sufficiently, to the development of amphetamine behavioral sensitization (Panayi et al., 2005). An increase in ventral midbrain DA release seems to be critical for this NT action. In fact, cocaine and amphetamine sensitization are augmented by compounds that increase extracellular catecholamine levels (Kalivas and Weber, 1988); and the stimulation of ventral midbrain NT receptors enhances neuronal DA firing and release (Kalivas and Duffy, 1990; Kalivas and Taylor, 1985). It is also possible that NT might produce psychostimulant sensitization by activating its receptors in the medial PFC, a brain area receiving NT afferents (Fatigati et al., 2000; Petrie et al., 2005; Rompré et al., 1998; Sesack and Pickel, 1992).

Although the available literature was focused on NT receptor antagonism in the development of drug sensitization, limited findings indicate the effects of NT receptor agonists on this phenomenon. Thus in rats, NT69L significantly reduced the initiation of amphetamine- and cocaine-induced behavioral sensitization (Fredrickson et al., 2003a, 2014). A local (intra-ventral pallidum) injection of the biologically active NT(8–13) fragments before systemic cocaine injection did not alter locomotor responses to a cocaine challenge, reducing the significance of the endogenous pallidal release of NT for the development of cocaine sensitization (Torregrassa and Kalivas, 2008).

## Expression of sensitization

Opposite to the controversial data obtained in studies on sensitization development, the results concerning the role of NT in the expression of psychostimulant drug sensitization consistently pro-

**Table 2(a).** Neurotensin and drug of abuse-evoked sensitization development.

Drug (dose range and administration route)	Drug of abuse (dose range and administration route)	Species (gender)	Sensitization change	Reference
Neurotensin analogues and agonists				
NT69L (1 mg/kg i.p.; once per week, for 5 weeks)	Nicotine (0.35 mg/kg s.c.; once per week, for 6 weeks)	SD rats (male)	↓	Fredrickson et al., 2003a
NT69L (1 mg/kg i.p.; for 15 days)	Nicotine (0.35 mg/kg s.c.; for 15 days)	SD rats (male)	↓	Fredrickson et al., 2003b
Neurotensin antagonists				
SR 48692 (40, 80, 160 mg/kg i.p.; day 1, 3, 5, 7)	Amph (1.5 mg/kg i.p.; day 1, 3, 5, 7) + Amph (0.75 mg/kg i.p.; day 14)	Long Evans rats (male)	↓	Romp�re and Perron, 2000
SR 48692 (0.1, 1 mg/kg i.p.; pretreatment from day 1 to 14)	Amph (0.5 or 1 mg/kg i.p.; day 1, 3, 5, 7 + day 14)	SD rats (male)	↓	Panayi et al., 2002
SR 48692 (80 µg/kg i.p. or p.o.; for 5 days) (pre-exposure)	Cocaine (15 mg/kg i.p.; every other day, for 6 days after pre-exposure)	SD rats (male)	↓	Horger et al., 1994
SR 48692 (80 µg/kg i.p.; co-treatment 2 and 4 days before test)	Cocaine (15 mg/kg i.p.; co-treatment 2 and 4 days before test + alone day of test)	SD rats (male)	0	Horger et al., 1994
SR 48692 (160, 320, 640 µg/kg i.p.; day 1, 3, 5, 7)	Morphine (5.0 mg/kg i.p.; day 1, 3, 5, 7) + morphine (2.5 mg/kg i.p.; day 14)	Long Evans rats (male)	↓	L�vesque et al., 2008
SR 142948A (5 pmol/side intra-VTA injection; day 1)	Amph (1 mg/kg s.c.; day 1 + day 8)	SD rats (male)	↓	Panayi et al., 2005
SR 142948A (0.03, 0.1, 0.3 mg/kg i.p.; day 1)	Amph (1 mg/kg s.c.; day 1 + day 8)	SD rats (male)	0	Panayi et al., 2005

↓ (arrow down): decrease; Amph: amphetamine; i.p.: intraperitoneal; NT: neurotensin; 0: no change; p.o.: per os or by mouth; s.c.: sub-cutaneous; SD: Sprague Dawley; VTA: ventral tegmental area.

**Table 2(b).** Neurotensin and drug of abuse-evoked sensitization expression.

Drug (dose range and administration route)	Drug of abuse (dose range and administration route)	Species (gender)	Sensitization change	Reference
Neurotensin analogues and agonists				
NT69L (1 mg/kg i.p.; pre-treatment to cocaine challenge)	Cocaine (20 mg/kg i.p.; for 4 days + challenge)	Wistar rats (male gender)	↓	Fredrickson et al., 2014
NT69L (1 mg/kg i.p.; pre-treatment to cocaine challenge)	Nicotine (0.35 mg/kg s.c.; for 7 days) + Cocaine (20 mg/kg i.p.; for 4 days) + Cocaine (20 mg/kg; i.p.)	Wistar rats (male gender)	↓	Fredrickson et al., 2014
Neurotensin antagonists				
SR 48692 (0.3 mg/kg; i.p.; day 2, 9, 16 after pre-treatment period)	Amph (2.0 mg/kg, i.p.; every other day for 13 days + day 2, 9, 16 after pre-treatment period)	Swiss mice (female gender)	↓	Costa et al., 2001
SR 48692 (0.3 mg/kg i.p.; for 7 days after amphetamine discontinuation)	Amph (2.0 mg/kg i.p.; every other day for 11 days in pre-treatment phase + day 8 after amphetamine discontinuation)	Swiss mice (male gender)	↓	Costa et al., 2007
SR 142948A (5 pmol/side, intra-VTA injection; day 8)	Amph (1 mg/kg; s.c.; day 1 + day 8)	SD rats (male gender)	0	Panayi et al., 2005

↓ (arrow down): decrease; Amph: amphetamine; i.p.: intraperitoneal; NT: neurotensin; 0: no change; s.c.: sub-cutaneous; SD: Sprague Dawley; VTA: ventral tegmental area.

vided evidence that the blockade of NT receptors decreases this process. In fact, the acute administration of SR48692, prior to amphetamine challenge doses, prevented the expression of amphetamine behavioral sensitization in rats that were pre-exposed to the psychostimulant (Costa et al., 2001). Furthermore, given daily after the amphetamine exposure period necessary to sensitize the

animals to the behavioral effects of the drug, SR48692 reverted the expression of amphetamine-induced sensitization (Costa et al., 2007). The same antagonist, chronically administered after a cocaine regimen during the withdrawal period, was effective in attenuating the expression of cocaine-elicited sensitization (Felszeghy et al., 2007).

**Table 2(c).** Neurotensin and drug of abuse-evoked sensitization, cross-sensitization.

Drug (dose range, administration route)	Drug of abuse (dose range, administration route)	Species (gender)	Change	References
Neurotensin and analogues				
NT (18 nmol/10 ml; i.c.v.; day 1, 3, 5, 7)	Amph (1 mg/kg i.p.; day 14)	Long Evans rats (male)	Cross-sensitization	Rompré, 1997
(D-Tyr11)-NT (18 nmol/10 ml i.c.v.; day 1, 3, 5, 7)	Amph (1 mg/kg i.p.; day 14)	Long Evans rats (male)	Cross-sensitization	Rompré, 1997
(D-Tyr11)-NT (18 nmol/10 ml i.c.v.; day 1, 3, 5, 7)	Cocaine (7.5 mg/kg i.p.; day 14)	Long Evans rats (male)	Cross-sensitization	Rompré and Bauco, 2006
(D-Tyr11)-NT (18 nmol/10 ml i.c.v.; day 1, 4, 7, 10)	Amph (0.75 mg/kg i.p.; day 17)	Long Evans rats (male)	Cross-sensitization	Rouibi and Rompré, 2014

Amph: amphetamine; i.c.v.: intracerebroventricular; i.p.: intraperitoneal; NT: neurotensin; s.c.: sub-cutaneous.

**Table 3.** NT and drug of abuse-evoked CPP.

Drug (dose range, administration route, schedule)	Drug of abuse (dose range, administration route, schedule)	Species (gender)	CPP change	Reference
NT antagonists				
Development				
SR 142948A (1 mg/kg i.p.; day 1, 3, 5)	MDMA (9 mg/kg i.p.; for 6 days, alternating with saline)	CD1 mice (male)	0	Marie-Claire et al., 2008
Expression				
SR 48692 (1 mg/kg i.p.; day 9–18)	Cocaine (15 mg/kg i.p.; day 2, 4, 6, 8 of conditioning phase + day 17)	SD rats (male)	↓	Felszeghy et al., 2007
SR 142948A (1 mg/kg i.p.; day 1, 3, 5 of conditioning phase + day 7)	MDMA (9 mg/kg i.p.; for 6 days of conditioning phase, alternating with saline)	CD1 mice (male)	↓	Marie-Claire et al., 2008

↓ (arrow down): decrease; CD1: cluster of differentiation 1; CPP: conditioned place preference; i.p.: intraperitoneal; MDMA: 3,4-methylenedioxymethamphetamine; NT: neurotensin; 0: no change; SD: Sprague Dawley.

When injected into the VTA, the other NT receptor antagonist, SR142948A, failed to alter the expression of amphetamine sensitization; however, this effect is in line with the accepted notion that the VTA by itself is not an anatomical substrate for expression of drug sensitization (Panayi et al., 2005). Even though most studies focus on the expression of behavioral sensitization to psychostimulant drugs mainly considered for the role of DA transmission, the imbalance between some neurotransmitter pathways, such as the glutamatergic and GABAergic ones, could contribute to this process (Pierce and Kalivas, 1997; Vanderschuren and Kalivas, 2000).

NT receptors are localized in most of the structures of the motive circuit; and that neuropeptide is known to interact with neuronal pathways involved in drug addiction (Liang et al., 2008; Picciotto and Corrigall, 2002). Thus, the NT-induced modulation of different neurotransmitter systems in these brain regions might be involved in the role that the peptide plays in the expression of psychostimulant sensitization; however, it is worth noting that systemic administration of both NT receptor agonists and antagonists have been reported to produce an attenuation of the expression of drug-elicited sensitization. This issue needs, therefore, to be deeply investigated by future studies, to better clarify the mechanisms through which the different NT receptor ligands exert these effects.

Finally, cross-sensitization between NT and psychostimulants has been observed. Specifically, it was shown that repeated,

intermittent i.c.v. injections of NT in rats produce sensitization to the behavioral stimulant effect of systemic amphetamine, while the more potent NT analogue D-Tyr[11]NT, repeatedly administered by i.c.v., increases both cocaine and amphetamine sensitization (Rompré, 1997; Rompré and Bauco, 2006). This action, at least in the case of amphetamine sensitization, was demonstrated to be either context-dependent or context-independent, based upon the pattern of locomotor activity (Rouibi and Rompré, 2014); and to be prevented by excitotoxic lesions of the PFC (Blackburn et al., 2004). Thus, given the long-lasting changes in responsiveness to psychostimulant drugs after repeated activation of the NT systems, it was reasonable to conclude that NT could act in the same neural mechanisms that induce some neuroadaptations related to drug sensitization; however, further investigation is required to better understand the exact role, the anatomical sites, and the functional and molecular mechanisms through which NT mediates these effects.

## Neurotensin and drug of abuse reward

Some evidence for the rewarding properties of NT and/or its ability to affect the reinforcing behaviors induced by drugs of abuse has been provided by studies using different experimental procedures, such as drug self-administration, drug preference and conditioned place preference (CPP) in rodents (Table 3 and Table 4).



**Table 4.** Neurotensin and drug of abuse-evoked self-administration.

Drug (dose range, administration route, schedule)	Drug of abuse (training dose range, administration route, reinforcement schedule)	Species (gender)	Self-administration change	Reference
<b>Maintenance</b>				
<b>NT</b>				
NT (4.2, 8.4 and 16.7 µg, total bilateral administration; intra-NAC injection)	Cocaine (0.75 mg/kg/infusion i.v.); SA FR5	Wistar rats (male)	0	Robledo et al., 1993
<b>NT analogues and agonists</b>				
NT69L (1.0 mg/kg i.p.); every 12 hours	Ethanol (3 to 6 to 10% v/v); 2-bottle choice	C57BL/6J mice (male)	↓	Lee et al., 2010; 2011
NT69L (1.0 mg/kg i.p.); every 12 hours	Ethanol (3 to 6 to 10% v/v); 2-bottle choice	NTS1 null mice (male)	0	Lee et al., 2010
NT69L (1.0 mg/kg i.p.); every 12 hours	Ethanol (3 to 6 to 10% v/v); 2-bottle choice	NTS2 null mice (male)	↓	Lee et al., 2011
NT69L (1 mg/kg i.p.)	Nicotine (0.03 mg/kg/infusion i.v.); SA FR1-FR5	SD rats (male)	↓	Boules et al., 2011
PD 149163 (0.5 mg/kg s.c.)	METH (0.06 mg/kg/infusion i.v.); SA FR1-FR5	SD rats (male)	↓	Frankel et al., 2010
<b>NT antagonists</b>				
SR 48692 (0.3 mg/kg); days 5-7 of SA	METH (0.06 mg/kg/infusion i.v.); SA FR1-FR5	SD rats (male)	0	Frankel et al., 2010
<b>Substitution</b>				
<b>NT analogue and agonists</b>				
PD 149163 (0.1 mg/kg/infusion i.v.)	METH (0.06 mg/kg/infusion i.v.); SA from FR1 to FR2 to FR3 to FR5	SD rats (male)	↓	Hanson et al., 2012

↓ (arrow down): decrease; Amph: amphetamine; FR: fixed ratio; i.p.: intraperitoneal; i.v.: intravenous; METH: methamphetamine; NAC: nucleus accumbens; NT: neurotensin; 0: no change; SA: self-administration; s.c.: sub-cutaneous; SD: Sprague Dawley.

### Drug self-administration

Early investigations using the self-administration paradigm demonstrated that rats performed operant tasks to obtain intra-VTA infusions of NT, thus suggesting that the peptide by itself exerts a primary positive reinforcement in this brain area (Glimcher et al., 1984, 1987). These findings are in line with previous results obtained in CPP experiments; however, when NT was injected into the NAC immediately before cocaine SA in the maintenance phase, no effect of the peptide on cocaine self-infusions was found, whereas the administration of the peptide in the same region attenuated the drug-elicited locomotor activity (Robledo et al., 1993). It is worth noting that, in the latter study, NT was administered into the NAC core that could be more involved in motor than in motivational aspects. In fact, the projections from the NAC core mainly innervate the ventromedial central pallidum, which participates in the indirect pathway to the subthalamic nucleus and the substantia nigra, pars reticulata. Instead, the projections from the NAC shell reach the dorsolateral ventral pallidum, which innervates the medio-dorsal thalamic nucleus, sending efferents to the prefrontal cortex (Fuxe et al., 2008), VTA and the lateral hypothalamus (Heimer et al., 1991; Kalivas and Miller, 1984; Zahm and Heimer, 1988, 1990). The microinjection of NT(8–13) into the ventral pallidum did not affect cocaine self-administration (Torregrossa and Kalivas, 2008).

On the other hand, the NT receptor agonist PD149163 blocked intravenous (i.v.) mephedrone self-administration (German et al., 2014) or i.v. methamphetamine (METH) self-injection in rats (Frankel et al., 2011; Hanson et al., 2012). Of note, in a substitution test of METH SA, the PD149163 was not self-administered by the animals, which excludes rewarding properties (Hanson et al., 2012). These authors proposed that self-administration extinction is associated with increases in NT release involving a DA D2 receptor mechanism in the NAC (Antonelli et al., 2007b; Borroto-Escuela et al., 2013).

Taken together, the data from combination and substitution SA paradigms provide evidence for the involvement of NT signaling in regulating psychostimulant consumption and suggest that the activation of NT receptors generally suppresses operant behaviors linked with drugs' SA.

It is well-known that the mesolimbic DA system, where NT also exists in its mesocortical component (Saint-Gelais et al., 2006; Von Euler et al., 1990) is the main anatomical substrate for the rewarding effects of drugs of abuse (McBride et al., 1999). NT is associated with inhibitory feedback actions on basal ganglia and limbic DA pathways, and elevated extracellular NT levels have been reported in both the NAC and dorsal striatum, after treatment with high doses of psychostimulants (German et al., 2014; Hanson et al., 2012). The mechanisms underlying the

**Table 5.** Neurotensin and drug preference.

Drug (dose range, administration route)	Drug of abuse (dose range, administration route)	Species (gender)	Change	References
NT analogue and agonists				
NT69L (1.0 mg/kg; i.p.)	Ethanol (3 to 6 to 10% v/v); 2-bottle choice	C57BL/ 6J mice (male)	↓	Lee et al., 2010, 2011
NT69L (1.0 mg/kg; i.p.)	Ethanol (3 to 6 to 10% v/v); 2-bottle choice	NTS1 null mice (male)	0	Lee et al., 2010
NT69L (1.0 mg/kg; i.p.)	Ethanol (3 to 6 to 10% v/v); 2-bottle choice	NTS2 null mice (male)	↓	Lee et al., 2011

↓ (arrow down): decrease; i.p.: intraperitoneal; NT: neurotensin; 0: no change; v/v: the percentage by volume.

ability of NT receptor agonists to reduce the reinforcing effects in i.v. self-administration and two drugs choice experiment seem complex, but are possibly related to the antagonistic interaction of the NTS1 receptor with the DA D2 receptor, reducing post-junctional DA D2 receptor signaling.

Based on results of the above-cited SA studies, which were focused mainly on the effects of psychostimulants, it seems likely that receptor-receptor interactions in the NAC NTS1-D2 heteroreceptor complexes contribute to counteract the reward properties of psychostimulants. Thus, an increased activity in the ventral striato-pallidal GABA neurons is obtained, which represents an anti-reward system.

### Conditioned place preference

CPP is widely used to study the motivational and reinforcing effects of both natural stimuli and drugs of abuse (Tzschentke, 2007). This paradigm is based upon the acquisition of preference for neutral environmental stimuli (conditioned or secondary reward) that were previously combined with a drug administration (primary reward). The CPP comprises an acquisition phase and an expression phase, in which drug-free animals are tested for their preference for the environment previously paired with the drug. It was reported that the acquisition and expression of this incentive learning are mediated by different neurochemical mechanisms. For example, the neurons involved in the expression and acquisition of CPP in the case of amphetamine and morphine are anatomically distinct, at least within the NAC (Fenu et al., 2006; Marie-Claire et al., 2008; Sellings and Clarke, 2003).

An early study reported that intra-VTA NT was associated with CPP acquisition and expression, thus suggesting that the peptide acts as a primary reinforcement in rats (Glimcher et al., 1984). More recently, it was demonstrated that NT displays positive reinforcing actions, when microinjected into the central nucleus of the amygdala and ventral pallidum, and that these effects were mediated by NTS1 receptors, because they were prevented by prior administration of selective receptor antagonists (Laszlo et al., 2010; Ollmann et al., 2015).

Other studies investigated the role of NT in drug of abuse-induced CPP. Pre-treatment with the NTS1/2 receptor blocker SR142948A did not modify the development of, but suppressed the expression of, MDMA-elicited CPP in mice (Marie-Claire et al., 2008); while chronic treatment with the more selective NTS1 receptor antagonist SR48692 blocked cocaine-induced CPP in rats (Felszeghy et al., 2007). These findings suggested

that endogenous NT might participate in some behaviors elicited by drugs of abuse and it appears to be mainly involved in the expression of psychostimulant-induced CPP. Interestingly, the mouse striatal NTS1 receptor mRNA levels were up-regulated when the MDMA-induced CPP expression was tested. Based on these results, it was proposed that NTS1 receptors may be involved in the behavioral consequences induced by the conditioned environmental reward, without directly mediating the reinforcing actions of drugs of abuse. Thus, NT could be a neural target for reward expectation (Marie-Claire et al., 2008); however, the rewarding properties of cocaine, as explored in the CPP paradigm, using NT knockout (KO) mice, are reported to be similar to those observed in wild-type (WT) animals, indicating that endogenous NT is not required for the cocaine-induced CPP and for the rewarding effect of the drug (Hall et al., 2012). Nevertheless, the authors did not exclude that NT could be involved in other aspects of cocaine-elicited CPP, such as the retention of CPP following cocaine conditioning (agreeing with Felszeghy et al., 2007) not explored in NT KO mice.

### Drug preference

The drug preference procedures have been mainly used to assess the involvement of NT in ethanol-mediated physiological and behavioral changes (Erwin and Su, 1989; Erwin et al., 1997; Luttinger et al., 1982; Widdowson, 1987); however, the functional role of NT and its receptors in ethanol preference has not been sufficiently established. The activation of NTS1 receptor signaling appears to be inversely correlated with ethanol preference (Table 5). In fact, the systemic administration of the NT analogue NT69L significantly reduced ethanol preference, in both WT and NTS2 receptor KO mice, in a 2-bottle choice experiment, while the NTS1 KO mice were insensitive to this effect (Lee et al., 2010, 2011). In line with these findings, rats that are alcohol-preferring show a lower concentration of PFC NT, in comparison with non-alcohol-preferring rats (Ehlers et al., 1999). In addition, either NTS1 or NTS2 KO mice display similar elevated ethanol intake, when compared to WT animals. It has been proposed that NTS1 receptors are involved in the effect of lower, ataxic doses of ethanol, whereas NTS2 receptors might be responsible for the effect of higher, hypnotic doses of ethanol (Lee et al., 2011). Concerning the possible mechanism of action, NT69L might prevent ethanol consumption through the modulation of both DAergic and glutamatergic systems that are implicated in ethanol addiction (Li

et al., 2011). Taken together, these data suggest that there is a potential therapeutic use of NT analogues in alcohol use disorder.

## Neurotensin and self-stimulation

Reward stimuli include, among others, electrical stimulations of some brain areas. In the paradigm of brain stimulation reward (BSR), rats rapidly learn an operant task, in order to electrically stimulate their own brain in specific sites that are mostly under control of DA neurotransmission. Consistent data show that BSR is modified by the administration of drugs of abuse, whose mechanisms of action were known to be related to a modulation of CNS DA transmission. Experiments using the self-stimulation procedure indicated that NT is involved in the control of behaviors motivated by positive reinforcement (Kempadoo et al., 2013; Rompré et al., 1992, 1995; Rompré and Boye, 1993). The evidence is supported by consistent data that indicate the anatomical and functional interactions between the DA brain reward system and the NT pathways seen in Figure 1 (Binder et al., 2001; Boules et al., 2013; Geisler et al., 2006; Saint-Gelais et al., 2006).

The microinjection of NT into the ventral mesencephalic region, produced a dose-dependent reduction in the stimulation frequency necessary to sustain the threshold levels of responding for BSR. The activation of ventral mesencephalon NT receptors induced DA neuron firing and the neurotransmitter release in limbic terminal fields; thus providing evidence that NT enhances reward-related activity in DA cells (Rompré et al., 1992), potentially through antagonistic NTS1-DA D2 autoreceptor interactions. Moreover, an augmentation of BRS is found when the peptide is i.c.v. injected, and this effect is associated with a significant time-dependent decrease in frequency threshold, in rats. These results provide evidence for psychostimulant-like effects of centrally-administered NT.

On the other hand, i.c.v. administration of the peptide was also found to suppress the maximal rate of response that is a typical action of neuroleptics. These results can be explained by the fact that i.c.v.-injected NT can also reach NAC and produce a reduction of postjunctional DA D2 receptor signaling, possibly via a NTS1-D2 receptor-receptor interaction (Borroto-Escuela et al., 2013). Of note, at a low dose NT tended to decrease the threshold; whereas at a higher dose, even a small increase was found immediately after the i.c.v. NT injection that might reach the NAC. Opposite effects of different doses of NT were also observed on spontaneous locomotion (Nouel et al. 1990), which may also be explained according to this proposal.

Recent work by Kempadoo et al. (2013) also reported that NTS1 receptors alter intracranial self-stimulation from the lateral hypothalamus to VTA in mice, as the selective antagonist SR48692 was found to decrease the amount of reinforced behavior, although maintaining lower levels of stimulation-seeking. The proposed mechanism involves NT-mediated enhancement of reward-related behavior via excitatory glutamate transmission on midbrain DA neurons, as well as by NT-mediated reduction in the D2 autoreceptor function in the VTA. Considering this evidence, it seems likely that the NT receptor antagonists might counteract human forms of pathological reward-seeking, among other mechanisms, through an action on the NTS1-D2 autoreceptor complex in the VTA DA neurons.

## Neurotensin and drug-seeking behavior

Recently, the role of NT in drug-seeking behavior and, specifically, in a relapse to drug-seeking following a prolonged drug-free period, has been investigated by a few authors (Table 6). In experimental models of drug-seeking, the animals were trained to self-administer a drug (reinforce); then the reinforcement was removed to extinguish this behavior, and finally, the drug-seeking was restored by the presentation of a stressor, a drug-associated cue (sound and/or light), or the drug itself (Torregrossa and Kalivas, 2008).

Using this paradigm, both NT and its analogue D-TYR[11] NT, injected i.c.v. prior to the reinstatement test in rats, were found to produce a robust reinstatement of cocaine-seeking behavior; and this effect was attenuated by a prior injection of a DA D1/D5 receptor antagonist. Interestingly, the same local pre-treatment with D-TYR[11]NT did not affect the reinstatement of sucrose-seeking in the rat.

The lack of efficacy of NT in non-drug (sucrose) reinstatement, in comparison to the cocaine-reinstatement, suggested the hypothesis that during the psychostimulant SA, certain neuronal adaptations in some brain regions lead NT to enhance the vulnerability to relapse, after a drug-free period (Lopak and Erb, 2005). Other data demonstrated that the active NT fragment NT (8–13), injected into the intra-ventral pallidum before the reinstatement test for cocaine-seeking, inhibited the cue-induced reinstatement (Torregrossa and Kalivas, 2008). This is likely related to NT fragment-induced GABA release from the striato-pallidal GABA terminals, via antagonistic NTS1-D2 interactions in those terminals. The same authors showed an unexpected augmentation of cocaine-primed reinstatement (Torregrossa and Kalivas, 2008). This profile of action is surprising, because both the cue- and the cocaine-induced reinstatements are mediated by ventral pallidum neurotransmission. At present, it is difficult to explain these results. It may be speculated that cocaine treatment has reorganized the postjunctional NTS1-DA D2 complexes of the striato-pallidal GABA neurons into producing facilitatory NTS1-DA D2 receptor-receptor interactions, which can explain the augmentation of the cocaine-induced reinstatement. Finally, only one dose of NT (8–13) was tested in the reinstatement experiments; thus, it cannot be excluded that other doses of the NT analogue might have different effects, so further experiments will be necessary to clarify this possibility. It is worth noting that when given systemically, but not when directly administered into the VP, the NTS1/2 receptor antagonist SR142948 blocked the cocaine-induced reinstatement, excluding the significance of ventral pallidum NT receptors to this behavioral effect (Torregrossa and Kalivas, 2008).

Taken together, these data indicated that NT systems seem to play a role in drug-seeking behavior, but additional studies are requested to deeply investigate the involvement of the neuropeptide in this model of drug relapse, and the exact mechanisms underlying the brain interactions between NT and other neurotransmitter systems during drug-induced seeking behavior.

## Neurotensin and drug withdrawal

Limited data show that NT may alter withdrawal symptoms following chronic exposure to some drugs of abuse. Thus, the NT analog NT(8–13) decreased behavioral signs of the withdrawal syndrome in rats that were repeatedly treated over 5 days with

**Table 6.** Neurotensin and reinstatement of drug-seeking behavior.

Drug (dose range, administration route)	Drug of abuse (training dose range, administration route, schedule of reinforcement)	Reinstatement	Species (gender)	Change in drug-seeking behavior	Reference
<b>Drug-induced</b>					
<b>NT analogues</b>					
NT(8–13) (3 nmol; intra-VP)	Cocaine (0.6 mg/kg/infusion i.v.); SA FR1	Cocaine (10, 30 mg/kg; i.p.)	SD rats (male)	↑	Torregrossa and Kalivas, 2008
<b>NT antagonists</b>					
SR 142948 (1 nmol; intra-VP)	Cocaine (0.6 mg/kg/infusion i.v.); SA FR1	Cocaine (10 mg/kg; i.p.)	SD rats (male)	0	Torregrossa and Kalivas, 2008
SR 142948 (10 µg/kg; i.p.)	Cocaine (0.6 mg/kg/infusion i.v.); SA FR1	Cocaine (10 mg/kg; i.p.)	SD rats (male)	↓	Torregrossa and Kalivas, 2008
<b>Cue-induced</b>					
<b>NT analogues</b>					
NT(8–13) (3 nmol; intra-VP)	Cocaine (0.6 mg/kg/infusion i.v.); SA FR1	Light + tone + syringe pump activation cues	SD rats (male)	↓	Torregrossa and Kalivas, 2008
<b>Other reinstatements</b>					
<b>NT and analogues</b>					
NT (15, 30 µg; i.c.v.)	cocaine (1.0 mg/kg/infusion i.v.); SA FR1	Lever pressing	Long Evans rats (male)	↑	Lopak et al., 2005
D-TYR[11]NT (15, 30 µg; i.c.v.)	cocaine (1.0 mg/kg/infusion i.v.); SA FR1	Lever pressing	Long Evans rats (male)	↑	Lopak et al., 2005

↓ (arrow down): decrease; FR: fixed ratio; i.c.v.: intracerebroventricular; i.p.: intraperitoneal; i.v.: intravenous; NT: neurotensin; 0: no change; SA: self-administered; s.c.: sub-cutaneous; SD: Sprague Dawley; VP: ventral pallidum area.

increasing doses of morphine, followed by the opioid receptor antagonist naloxone. Such behavioral reductions were due to NT(8–13) restoration of decreased DA and serotonin turnover in brain areas (Konstantinopolskii et al., 2013). Another report showed no effects of NT (i.c.) on ethanol-withdrawal tremor and on audiogenic-seizure susceptibility in ethanol (Frye et al., 1981). The lack of effects during withdrawal from chronic ethanol on NT-like immunoreactivity (Wachi et al., 1996) and on NT binding sites (Campbell and Erwin, 1993) supports the above negative behavioral data.

On the other hand, 14 days of repeated cocaine injections and its early (24 hours) or late (8 days) withdrawal enhanced NT-like immunoreactivity in the substantia nigra; however, the degree of this increase was dependent on the mode of drug administration: Subcutaneous injection versus continuous minipumps (Cain et al., 1993). Changes after cocaine injections were also reported in rat brains processed for a NT receptor autoradiography, with significant changes of binding in subcortical areas and the PFC (Pilotte et al., 1991). Up till now, there has been no behavioral proof for NT-cocaine (or other drugs of abuse) interactions during drug withdrawal.

## Conclusions

The mesolimbic and mesocortical DA pathways are highly implicated in the psychomotor stimulant and reinforcing effects of

drugs of abuse. The above-reported studies strongly suggest that NT signaling is one of the specific neurochemical mechanisms for the modulation of the rewarding activity of cocaine and other drugs of abuse. Nevertheless, controversial results exist. At the present, the precise molecular mechanisms by which NT exerts its role in drug addiction remain to be elucidated. Furthermore, it is quite surprisingly the total lack of notion to date about the potential role of the peptide in stress-induced reinstatement of drug-seeking behavior, a very important aspect of relapse that is due to the recognized role of NT in stress regulation.

In contrast to what is the case after treatment with typical antipsychotic drugs, the psychostimulants enhance NT levels, mainly in subpopulations of the striato-nigral GABA pathways (Merchant, 1994) that are regulated primarily by the DA D1 receptors, although increases in NT levels in other brain regions have been also described. An increase in NT levels and the consequent NTS1 receptor stimulation appear to be essential for c-fos expression; and thus, activation of subpopulations of the striato-nigral GABA pathway (Fadel et al., 2006) that may be part of the reward pathways, especially when originating from the NAC. Furthermore, it could be possible that the psychostimulant-induced NT levels in the cell body-dendritic regions of these nerve cells would allow the peptide, through extrasynaptic volume transmission, to diffuse and reach the glutamate and DA terminals. Here, the NT peptides would inhibit DA D2 receptor signaling, via the 'antagonistic' NTS1-DA D2 receptor-receptor

interaction in the striatal and accumbal glutamate and DA (only dorsal striatal) terminals, leading to increased DA and glutamate release. This increased synaptic glutamate and extra-synaptic DA release may enhance activation of accumbal-VTA GABA pathways that are participating in the reward circuits that are activated by drugs of abuse; however, released NT in the NAC, via NTS1-DA D2 receptor-receptor interactions in the GABA neurons and their glutamate afferents, which project from the NAC to the ventral pallidum, can also activate the anti-reward circuit. In other words, the NT-induced alterations in the balance of activity in the reward and the anti-reward systems will determine how NT mechanisms contribute to counteracting the development of psychostimulant sensitization and possibly, cocaine use disorder.

NTS1 receptors are also localized in the VTA, where their activation may antagonize DA D2 autoreceptor functions and increase glutamate drive, thus enhancing drug abuse by increasing activity in the meso-limbic DA neurons mediating reward. Enhanced NTS1-NMDA receptor interactions in the VTA DA cells also lead to an increased activation of the mesolimbic DA reward neurons.

Based on the current preclinical research, there is no doubt that NT and its heteroreceptor complexes play a significant role in the modulation of substance use disorder. Further research is highly warranted, to understand the mechanisms involved in the interactions between NT, its heteroreceptor complexes including the NTS1-D2 heterocomplex and drugs of abuse, in the brain circuits of reward and anti-reward. Finally, it should be considered that only male animals were used in the previous studies that aimed to investigate the effects of NT in drug addiction. For these animal models to be most relevant to drug abuse in humans, the effects of NT compounds in female rodents will also need to be investigated.

### Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

### Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This research was partly supported by the Swedish Research Council (grant number 04X-715 to KF).

### References

- Agnati LF, Fuxe K, Benfenati F, et al. (1983) Neurotensin in vitro markedly reduces the affinity in subcortical limbic 3H-N-propylmorphine binding sites. *Acta Physiol Scand* 119: 459–461.
- Antonelli T, Fuxe K, Tomasini MC, et al. (2007a) Neurotensin receptor mechanisms and its modulation of glutamate transmission in the brain: Relevance for neurodegenerative diseases and their treatment. *Prog Neurobiol* 83: 92–109.
- Antonelli T, Tomasini MC, Fournier J, et al. (2008) Neurotensin receptor involvement in the rise of extracellular glutamate levels and apoptotic nerve cell death in primary cortical cultures after oxygen and glucose deprivation. *Cereb Cortex* 18: 1748–1757.
- Antonelli T, Tomasini MC, Fuxe K, et al. (2007b) Receptor-receptor interactions as studied with microdialysis. Focus on NTR/D2 interactions in the basal ganglia. *J Neural Transm* 114: 105–113.
- Berger B, Gaspar P and Verney C (1992) Colocalization of neurotensin in the mesocortical dopaminergic system. Restricted regional and laminar distribution in rat, lack of colocalization in human. *Ann NY Acad Sci* 668: 307–310.
- Betancur C, Cabrera R, De Kloet ER, et al. (1998) Role of endogenous neurotensin in the behavioral and neuroendocrine effects of cocaine. *Neuropsychopharmacology* 19: 322–332.
- Binder EB, Kinkead B, Owens MJ, et al. (2001) Neurotensin and dopamine interactions. *Pharmacol Rev* 53: 453–486.
- Blackburn A, Dewar K, Bauco P, et al. (2004) Excitotoxic lesions of the prefrontal cortex attenuate the potentiation of amphetamine-induced locomotion by repeated neurotensin receptor activation. *Brain Res* 998: 184–193.
- Borrito-Escuela DO, Ravani A, Tarakanov AO, et al. (2013) Dopamine D2 receptor signaling dynamics of dopamine D2-neurotensin 1 receptor heteromers. *Biochem Biophys Res Commun* 435: 140–146.
- Bose P, Rompré PP and Warren RA (2015) Neurotensin enhances glutamatergic EPSCs in VTA neurons by acting on different neurotensin receptors. *Peptides* 73: 43–50.
- Botto JM, Guillemare E, Vincent JP, et al. (1997) Effects of SR 48692 on neurotensin-induced calcium-activated chloride currents in the *Xenopus* oocyte expression system: Agonist-like activity on the levocabastine-sensitive neurotensin receptor and absence of antagonist effect on the levocabastine insensitive neurotensin receptor. *Neurosci Lett* 223:193–196.
- Boules M, Fredrickson P and Richelson E (2006) Bioactive analogs of neurotensin: Focus on CNS effects. *Peptides* 27: 2523–2533.
- Boules M, Li Z, Smith K, et al. (2013) Diverse roles of neurotensin agonists in the central nervous system. *Front Endocrinol* 4: 36.
- Boules M, McMahan B, Wang R, et al. (2003) Selective tolerance to the hypothalamic and anticataleptic effects of a neurotensin analog that crosses the blood-brain barrier. *Brain Res* 987: 39–48.
- Boules M, Oliveros A, Liang Y, et al. (2011) A neurotensin analog, NT69L, attenuates intravenous nicotine self-administration in rats. *Neuropeptides* 45: 9–16.
- Boules M, Shaw A, Fredrickson P, et al. (2007) Neurotensin agonists: Potential in the treatment of schizophrenia. *CNS Drugs* 21: 13–23.
- Boules M, Warrington L, Fauq A, et al. (2001) A novel neurotensin analog blocks cocaine- and D-amphetamine-induced hyperactivity. *Eur J Pharmacol* 426: 73–76.
- Boules MM, Fredrickson P, Muehlmann AM, et al. (2014) Elucidating the role of neurotensin in the pathophysiology and management of major mental disorders. *Behav Sci* 4: 125–153.
- Cáceda R, Binder EB, Kinkead B, et al. (2012) The role of endogenous neurotensin in psychostimulant-induced disruption of prepulse inhibition and locomotion. *Schizophr Res* 136: 88–95.
- Cáceda R, Kinkead B and Nemeroff CB (2006) Neurotensin: Role in psychiatric and neurological diseases. *Peptides* 27: 2385–2404.
- Campbell AD and Erwin VG (1993) Chronic ethanol administration downregulates neurotensin receptors in long- and short-sleep mice. *Pharmacol Biochem Behav* 45: 95–106.
- Carraway R and Leeman SE (1973) The isolation of a new hypotensive peptide, neurotensin, from bovine hypothalamus. *J Biol Chem* 248: 6854–6861.
- Carraway R and Leeman SE (1976) Characterization of radioimmunoassayable neurotensin in the rat. Its differential distribution in the central nervous system, small intestine and stomach. *J Biol Chem* 251: 7045–7052.
- Casti P, Marchese G, Casu G, et al. (2004) Blockade of neurotensin receptors affects differently hypo-locomotion and catalepsy induced by haloperidol in mice. *Neuropharmacology* 47: 128–135.
- Costa FG, Frussa-Filho R, Canteras NS, et al. (2007) Blockade of neurotensin receptors during amphetamine discontinuation indicates individual variability. *Neuropeptides* 41: 83–91.
- Costa FG, Frussa-Filho R and Felicio LF (2001) The neurotensin receptor antagonist, SR48692, attenuates the expression of amphetamine-induced behavioural sensitisation in mice. *Eur J Pharmacol* 428: 97–103.
- Dobner PR, Barber DL, Villa-Komaroff L, et al. (1987) Cloning and sequence analysis of cDNA for the canine neurotensin/neuromedin N precursor. *Proc Natl Acad Sci USA* 84: 3516–3520.

- Ehlers CL, Somes C, Li TK, et al. (1999) Neurotensin studies in alcohol naive, preferring and non-preferring rats. *Neuroscience* 93: 227–236.
- Elliott PJ and Nemeroff CB (1986) Repeated neurotensin administration in the ventral tegmental area: Effects on baseline and D-amphetamine-induced locomotor activity. *Neurosci Lett* 68: 239–244.
- Erwin VG and Su NC (1989) Neurotensin and ethanol interactions on hypothermia and locomotor activity in LS and SS mice. *Alcohol Clin Exp Res* 13: 91–94.
- Erwin VG, Markel PD, Johnson TE, et al. (1997) Common quantitative trait loci for alcohol-related behaviors and central nervous system neurotensin measures: Hypnotic and hypothermic effects. *J Pharmacol Exp Ther* 280: 911–918.
- Fadel J, Dobner PR and Deutch AY (2006) Amphetamine-elicited striatal Fos expression is attenuated in neurotensin null mutant mice. *Neurosci Lett* 402: 97–101.
- Fatigati MD, Anderson RM and Rompré P (2000) Effects of prefrontal cortex microinjection of neurotensin-(8-13) on midbrain dopamine and non-dopamine cell firing. *Brain Res* 876: 196–200.
- Feifel D, Melendez G, Murray RJ, et al. (2008) The reversal of amphetamine-induced locomotor activation by a selective neurotensin-1 receptor agonist does not exhibit tolerance. *Psychopharmacology* 200: 197–203.
- Felszeghy K, Espinosa JM, Scarna H, et al. (2007) Neurotensin receptor antagonist administered during cocaine withdrawal decreases locomotor sensitization and conditioned place preference. *Neuropsychopharmacology* 32: 2601–2610.
- Fenu S, Spina L, Rivas E, et al. (2006) Morphine-conditioned single-trial place preference: Role of nucleus accumbens shell dopamine receptors in acquisition, but not expression. *Psychopharmacology* 187: 143–153.
- Ferraro L, Beggiano S, Borroto-Escuela DO, et al. (2014) Neurotensin NT1-dopamine D2 receptor-receptor interactions in putative receptor heteromers: Relevance for Parkinson's disease and schizophrenia. *Curr Protein Pept Sci* 15: 681–690.
- Ferraro L, Tomasini MC, Beggiano S, et al. (2009) Emerging evidence for neurotensin receptor 1 antagonists as novel pharmaceuticals in neurodegenerative disorders. *Mini Rev Med Chem* 9: 1429–1438.
- Ferraro L, Tomasini MC, Fuxe K, et al. (2007) Mesolimbic dopamine and cortico-accumbens glutamate afferents as major targets for the regulation of the ventral striato-pallidal GABA pathways by neurotensin peptides. *Brain Res Rev* 55: 144–154.
- Ferraro L, Tomasini MC, Mazza R, et al. (2008) Neurotensin receptors as modulators of glutamatergic transmission. *Brain Res Rev* 58: 365–373.
- Filip M and Siwanowicz J (2001) Implication of the nucleus accumbens shell, but not core, in the acute and sensitizing effects of cocaine in rats. *Pol J Pharmacol* 53: 459–466.
- Frankel PS, Hoonakker AJ, Alburges ME, et al. (2011) Effect of methamphetamine self-administration on neurotensin systems of the basal ganglia. *J Pharmacol Exp Ther* 336: 809–815.
- Fredrickson P, Boules M, Stennett B, et al. (2014) Neurotensin agonist attenuates nicotine potentiation to cocaine sensitization. *Behav Sci* 4: 42–52.
- Fredrickson P, Boules M, Yerbury S, et al. (2003a) Novel neurotensin analog blocks the initiation and expression of nicotine-induced locomotor sensitization. *Brain Res* 979: 245–248.
- Fredrickson P, Boules M, Yerbury S, et al. (2003b) Blockade of nicotine-induced locomotor sensitization by a novel neurotensin analog in rats. *Eur J Pharmacol* 458: 111–118.
- Frye GD, Luttinger D, Nemeroff CB, et al. (1981) Modification of the actions of ethanol by centrally active peptides. *Peptides* 2: S99–106.
- Fuxe K, O'Connor WT, Antonelli T, et al. (1992) Evidence for a substrate of neuronal plasticity based on pre- and postsynaptic neurotensin-dopamine receptor interactions in the neostriatum. *Proc Natl Acad Sci USA* 89: 5591–5595.
- Fuxe K, Marcellino D, Rivera A, et al. (2008) Receptor-receptor interactions within receptor mosaics. Impact on neuropsychopharmacology. *Brain Res Rev* 58: 415–452.
- Geisler S, Bérod A, Zahm DS, et al. (2006) Brain neurotensin, psychostimulants and stress-emphasis on neuroanatomical substrates. *Peptides* 27: 2364–2384.
- German CL, Hoonakker AH, Fleckenstein AE, et al. (2014) Mephedrone alters basal ganglia and limbic neurotensin systems. *J Neurochem* 130: 402–407.
- Glimcher PW, Giovino AA and Hoebel BG (1987) Neurotensin self-injection in the ventral tegmental area. *Brain Res* 403: 147–150.
- Glimcher PW, Margolin DH, Giovino AA, et al. (1984) Neurotensin: A new 'reward peptide'. *Brain Res* 291: 119–124.
- Hall FS, Centeno M, Perona MT, et al. (2012) Effects of neurotensin gene knockout in mice on the behavioral effects of cocaine. *Psychopharmacology* 219: 35–45.
- Hanson GR, Hoonakker AJ, Alburges ME, et al. (2012) Response of limbic neurotensin systems to methamphetamine self-administration. *Neuroscience* 203: 99–107.
- Hedou G, Feldon J and Heidbreder CA (1999) Effects of cocaine on dopamine in subregions of the rat prefrontal cortex and their efferents to subterritories of the nucleus accumbens. *Eur J Pharmacol* 372: 143–155.
- Heimer L, Zahm DS, Churchill L, et al. (1991) Specificity in the projection patterns of accumbal core and shell in the rat. *Neuroscience* 41: 89–125.
- Hertel P, Byskov L, Didriksen M, et al. (2001) Induction of tolerance to the suppressant effect of the neurotensin analogue NT69L on amphetamine-induced hyperactivity. *Eur J Pharmacol* 422: 77–81.
- Horger BA, Taylor JR, Elsworth JD, et al. (1994) Preexposure to, but not cotreatment with, the neurotensin antagonist SR 48692 delays the development of cocaine sensitization. *Neuropsychopharmacology* 11: 215–222.
- Iversen LL, Nicoll RA and Vale WW (1978) Neurobiology of peptides. *Neurosci Res Program Bull* 16: 209–370.
- Kalivas PW and Duffy P (1990) Effect of acute and daily neurotensin and enkephalin treatments on extracellular dopamine in the nucleus accumbens. *J Neurosci* 10: 2940–2949.
- Kalivas PW and Miller JS (1984) Neurotensin neurons in the ventral tegmental area project to the medial nucleus accumbens. *Brain Res* 300: 157–160.
- Kalivas PW and Taylor S (1985) Behavioral and neurochemical effect of daily injection with neurotensin into the ventral tegmental area. *Brain Res* 358: 70–76.
- Kalivas PW and Weber B (1988) Amphetamine injection into the ventral mesencephalon sensitizes rats to peripheral amphetamine and cocaine. *J Pharmacol Exp Ther* 245: 1095–1102.
- Kalivas PW, Burgess SK, Nemeroff CB, et al. (1983) Behavioral and neurochemical effects of neurotensin microinjection into the ventral tegmental area of the rat. *Neuroscience* 8: 495–505.
- Kalivas PW, Nemeroff CB and Prange AJ, Jr (1984) Neurotensin microinjection into the nucleus accumbens antagonizes dopamine-induced increase in locomotion and rearing. *Neuroscience* 11: 919–930.
- Katsanos GS, Anogianaki A, Castellani ML, et al. (2008) Biology of neurotensin: Revisited study. *Int J Immunopathol Pharmacol* 21: 255–259.
- Kempadoo KA, Tourino C, Cho SL, et al. (2013) Hypothalamic neurotensin projections promote reward by enhancing glutamate transmission in the VTA. *J Neurosci* 33: 7618–7626.
- Kinkead B and Nemeroff CB (2004) Neurotensin, schizophrenia and antipsychotic drug action. *Int Rev Neurobiol* 59: 327–349.
- Kitabgi P (2006) Prohormone convertases differentially process pro-neurotensin/neuromedin N in tissues and cell lines. *J Mol Med* 84: 628–634.
- Kitabgi P (2010) Neurotensin and neuromedin N are differentially processed from a common precursor by prohormone convertases in tissues and cell lines. *Results Probl Cell Differ* 50: 85–96.
- Kitabgi P, Carraway R and Leeman SE (1976) Isolation of a tridecapeptide from bovine intestinal tissue and its partial characterization as neurotensin. *J Biol Chem* 251: 7053–7058.

- Kitabgi P, De Nadai F, Rovère C, et al. (1992) Biosynthesis, maturation, release, and degradation of neurotensin and neuromedin N. *Ann NY Acad Sci* 668: 30–42.
- Kitabgi P, Hervé D, Studler JM, et al. (1989) Neurotensin/dopamine interactions. *Encephale Spec No*: 91–94.
- Konstantinopol'skii MA, Cherniakova IV, Kudrin VS, et al. (2013) [Neurotensin NT (8–13) dipeptide analog dilept increases the pain threshold and decreases the severity of morphine withdrawal syndrome in rats]. *Eksp Klin Farmakol* 76: 6–11.
- LaCrosse AL and Olive MF (2013) Neuropeptide systems and schizophrenia. *CNS Neurol Disord Drug Targets* 12: 619–632.
- László K, Tóth K, Kertes E, et al. (2010) The role of neurotensin in positive reinforcement in the rat central nucleus of amygdala. *Behav Brain Res* 208: 430–435.
- Lee MR, Hinton DJ, Song JY, et al. (2010) Neurotensin receptor Type 1 regulates ethanol intoxication and consumption in mice. *Pharmacol Biochem Behav* 95: 235–241.
- Lee MR, Hinton DJ, Unal SS, et al. (2011) Increased ethanol consumption and preference in mice lacking neurotensin receptor Type 2. *Alcohol Clin Exp Res* 35: 99–107.
- Lévesque K, Lamarche C and Rompré PP (2008) Evidence for a role of endogenous neurotensin in the development of sensitization to the locomotor stimulant effect of morphine. *Eur J Pharmacol* 594: 132–138.
- Li XM, Ferraro L, Tanganelli S, O'Connor WT, et al. (1995) Neurotensin peptides antagonistically regulate postsynaptic dopamine D2 receptors in rat nucleus accumbens: A receptor binding and microdialysis study. *J Neural Transm Gen Sect* 102: 125–137.
- Liang Y, Boules M, Shaw AM, et al. (2008) Effect of a novel neurotensin analog, NT69L, on nicotine-induced alterations in monoamine levels in rat brain. *Brain Res* 1231: 6–15.
- Lopak V and Erb S (2005) Activation of central neurotensin receptors reinstates cocaine seeking in the rat: Modulation by a D1/D5, but not D2/D3, receptor antagonist. *Psychopharmacology* 182: 297–304.
- Luttinger D, Nemeroff CB and Prange AJ, Jr (1982) The effects of neuropeptides on discrete-trial conditioned avoidance responding. *Brain Res* 237: 183–192.
- McBride WJ, Murphy JM and Ikemoto S (1999) Localization of brain reinforcement mechanisms: Intracranial self-administration and intracranial place-conditioning studies. *Behav Brain Res* 101: 129–152.
- Marie-Claire C, Palminteri S, Romualdi P, et al. (2008) Effects of the selective neurotensin antagonist SR 142948A on 3,4-methylenedioxymethamphetamine-induced behaviours in mice. *Neuropharmacology* 54: 1107–1111.
- Mazella J and Vincent JP (2006) Functional roles of the NTS2 and NTS3 receptors. *Peptides* 27: 2469–2475.
- Meisenberg G and Simmons WH (1985) Motor hypoactivity induced by neurotensin and related peptides in mice. *Pharmacol Biochem Behav* 22: 189–193.
- Merchant KM, Dobie DJ, Filloux FM, et al. (1994) Effects of chronic haloperidol and clozapine treatment on neurotensin and c-fos mRNA in rat neostriatal subregions. *J Pharmacol Exp Ther* 271: 460–471.
- Minamino N, Kangawa K and Matsuo H (1984) Neuromedin N: A novel neurotensin-like peptide identified in porcine spinal cord. *Biochem Biophys Res Commun* 122: 542–549.
- Myers RM, Shearman JW, Kitching MO, et al. (2009) Cancer, chemistry and the cell: Molecules that interact with the neurotensin receptors. *ACS Chem Biol* 4: 503–525.
- Nemeroff CB (1980) Neurotensin: Perchance an endogenous neuroleptic? *Biol Psychiatry* 15: 283–302.
- Nemeroff CB, Luttinger D, Hernandez DE, et al. (1983) Interactions of neurotensin with brain dopamine systems: Biochemical and behavioral studies. *J Pharmacol Exp Ther* 225: 337–345.
- Noel D, Dubuc I, Kitabgi P, et al. (1990) Centrally administered [D-Trp11] neurotensin, as well as neurotensin protected from inactivation by thiorphan, modifies locomotion in rats in a biphasic manner. *Peptides* 11: 551–555.
- Ollmann T, Péczely L, László K, et al. (2015) Positive reinforcing effect of neurotensin microinjection into the ventral pallidum in conditioned place preference test. *Behav Brain Res* 278: 470–475.
- Panayi F, Colussi-Mas J, Lambás-Señas L, et al. (2005) Endogenous neurotensin in the ventral tegmental area contributes to amphetamine behavioral sensitization. *Neuropsychopharmacology* 30: 871–879.
- Panayi F, Dorso E, Lambás-Señas L, et al. (2002) Chronic blockade of neurotensin receptors strongly reduces sensitized, but not acute, behavioral response to D-amphetamine. *Neuropsychopharmacology* 26: 64–74.
- Pelaprat D (2006) Interactions between neurotensin receptors and G proteins. *Peptides* 27: 2476–2487.
- Petrie KA, Schmidt D, Bubser M, et al. (2005) Neurotensin activates GABAergic interneurons in the prefrontal cortex. *J Neurosci* 25: 1629–1636.
- Phillips TJ and Shen EH (1996) Neurochemical bases of locomotion and ethanol stimulant effects. *Int Rev Neurobiol* 39: 243–282.
- Picciozzo MR and Corrigall WA (2002) Neuronal systems underlying behaviors related to nicotine addiction: Neural circuits and molecular genetics. *J Neurosci* 22: 3338–3341.
- Pierce RC and Kalivas PW (1997) A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants. *Brain Res Brain Res Rev* 25: 192–216.
- Pilotte NS, Mitchell WM, Sharpe LG, et al. (1991) Chronic cocaine administration and withdrawal of cocaine modify neurotensin binding in rat brain. *Synapse* 9:111–120.
- Richard F, Barroso S, Martinez J, et al. (2001) Agonism, inverse agonism, and neutral antagonism at the constitutively active human neurotensin receptor 2. *Mol Pharmacol* 60: 1392–1398.
- Richelson E, Boules M and Fredrickson P (2003) Neurotensin agonists: Possible drugs for treatment of psychostimulant abuse. *Life Sci* 73: 679–690.
- Robinson TE and Berridge KC (1993) The neural basis of drug craving: An incentive sensitization theory of addiction. *Brain Res Brain Res Rev* 18: 247–291.
- Robledo P, Maldonado R and Koob GF (1993) Neurotensin injected into the nucleus accumbens blocks the psychostimulant effects of cocaine but does not attenuate cocaine self-administration in the rat. *Brain Res* 622: 105–112.
- Rompré PP (1995) Psychostimulant-like effect of central microinjection of neurotensin on brain stimulation reward. *Peptides* 16: 1417–1420.
- Rompré PP (1997) Repeated activation of neurotensin receptors sensitizes to the stimulant effect of amphetamine. *Eur J Pharmacol* 328: 131–134.
- Rompré PP and Baucó P (2006) Neurotensin receptor activation sensitizes to the locomotor stimulant effect of cocaine: A role for NMDA receptors. *Brain Res* 1085: 77–86.
- Rompré PP and Boye SM (1993) Opposite effects of mesencephalic microinjections of cholecystokinin octapeptide and neurotensin-(1-13) on brain stimulation reward. *Eur J Pharmacol* 232: 299–303.
- Rompré PP and Perron S (2000) Evidence for a role of endogenous neurotensin in the initiation of amphetamine sensitization. *Neuropharmacology* 39: 1880–1892.
- Rompré PP, Baucó P and Gratton A (1992) Facilitation of brain stimulation reward by mesencephalic injections of neurotensin-(1-13). *Eur J Pharmacol* 211: 295–303.
- Rompré PP, Boye SM and Moisan J (1998) Activation of neurotensin receptors in the prefrontal cortex stimulates midbrain dopamine cell firing. *Eur J Pharmacol* 341: 169–172.
- Rostène W, Brouard A, Dana C, et al. (1992) Interaction between neurotensin and dopamine in the brain. Morphofunctional and clinical evidence. *Ann NY Acad Sci* 668: 217–231.
- Rouibi K and Rompré PP (2014) Role of context in neurotensin-induced sensitization to the locomotor stimulant effect of amphetamine. *Peptides* 58: 103–107.

- Saint-Gelais F, Jomphe C and Trudeau LE (2006) The role of neurotensin in central nervous system pathophysiology: What is the evidence? *J Psychiatry Neurosci* 31: 229–245.
- Sarhan S, Hitchcock JM, Grauffel CA, et al. (1997) Comparative anti-psychotic profiles of neurotensin and a related systemically active peptide agonist. *Peptides* 18: 1223–1227.
- Sellings LH and Clarke PB (2003) Segregation of amphetamine reward and locomotor stimulation between nucleus accumbens medial shell and core. *J Neurosci* 23: 6295–6303.
- Sesack SR and Pickel VM (1992) Prefrontal cortical efferents in the rat synapse on unlabeled neuronal targets of catecholamine terminals in the nucleus accumbens septi and on dopamine neurons in the ventral tegmental area. *J Comp Neurol* 320: 145–160.
- Shi WS and Bunney BS (1990) Neurotensin attenuates dopamine D2 agonist quinpirole-induced inhibition of midbrain dopamine neurons. *Neuropharmacology* 29: 1095–1097.
- Shilling PD, Richelson E and Feifel D (2003) The effects of systemic NT69L, a neurotensin agonist, on baseline and drug-disrupted pre-pulse inhibition. *Behav Brain Res* 143: 7–14.
- Skoog KM, Cain ST and Nemeroff CB (1986) Centrally administered neurotensin suppresses locomotor hyperactivity induced by d-amphetamine but not by scopolamine or caffeine. *Neuropharmacology* 25: 777–782.
- Steinberg R, Brun P, Souilhac J, et al. (1995) Neurochemical and behavioural effects of neurotensin vs D-Tyr11 neurotensin on mesolimbic dopaminergic function. *Neuropeptides* 28: 43–50.
- Steketee JD and Kalivas PW (2011) Drug wanting: Behavioral sensitization and relapse to drug-seeking behavior. *Pharmacol Rev* 63: 348–365.
- Tanganelli S, Antonelli T, Tomasini MC, et al. (2012) Relevance of dopamine D(2)/neurotensin NT1 and NMDA/neurotensin NT1 receptor interaction in psychiatric and neurodegenerative disorders. *Curr Med Chem* 19: 304–316.
- Tanganelli S, O'Connor WT, Ferraro L, et al. (1994) Facilitation of GABA release by neurotensin is associated with a reduction of dopamine release in rat nucleus accumbens. *Neuroscience* 60: 649–657.
- Torregrossa MM and Kalivas PW (2008) Neurotensin in the ventral pallidum increases extracellular gamma-aminobutyric acid and differentially affects cue- and cocaine-primed reinstatement. *J Pharmacol Exp Ther* 325: 556–566.
- Tyler-McMahon BM, Boules M and Richelson E (2000) Neurotensin: Peptide for the next millennium. *Regul Pept* 93: 125–136.
- Tzschentke TM (2007) Measuring reward with the conditioned place preference (CPP) paradigm: Update of the last decade. *Addict Biol* 12: 227–462.
- Tzschentke TM and Schmidt WJ (2003) Glutamatergic mechanisms in addiction. *Mol Psychiatry* 8: 373–382.
- Vadnie CA, Hinton DJ, Choi S, et al. (2014) Activation of neurotensin receptor Type 1 attenuates locomotor activity. *Neuropharmacology* 85: 482–492.
- Vanderschuren LJ and Kalivas PW (2000) Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: A critical review of preclinical studies. *Psychopharmacology* 151: 99–120.
- Vincent JP, Mazella J and Kitabgi P (1999) Neurotensin and neurotensin receptors. *Trends Pharmacol Sci* 20: 302–309.
- Vita N, Oury-Donat F, Chalou P, et al. (1998). Neurotensin is an antagonist of the human neurotensin NT2 receptor expressed in Chinese hamster ovary cells. *Eur J Pharmacol* 360: 265–272.
- Von Euler G (1991) Biochemical characterization of the intramembrane interaction between neurotensin and dopamine D2 receptors in the rat brain. *Brain Res* 561: 93–98.
- Von Euler G and Fuxe K (1987) Neurotensin reduces the affinity of D2-dopamine receptors in rat striatal membranes. *Acta Physiol Scand* 131: 625–626.
- Von Euler G, Meister B, Hökfelt T, et al. (1990) Intraventricular injection of neurotensin reduces dopamine D2 agonist binding in rat forebrain and intermediate lobe of the pituitary gland. Relationship to serum hormone levels and nerve terminal coexistence. *Brain Res* 531: 253–262.
- Von Euler G, Van der Ploeg I, Fredholm BB, et al. (1991) Neurotensin decreases the affinity of dopamine D2 agonist binding by a G protein-independent mechanism. *J Neurochem* 56: 178–183.
- Wachi M, Fujimaki M, Nakamura H, et al. (1996) Effects of ethanol administration on brain neurotensin-like immunoreactivity in rats. *Acta Neurol Scand* 93: 211–214.
- Werkman TR, Kruse CG, Nievelstein H, et al. (2000) Neurotensin attenuates the quinpirole-induced inhibition of the firing rate of dopamine neurons in the rat substantia nigra pars compacta and the ventral tegmental area. *Neuroscience* 95: 417–423.
- Widdowson PS (1987) The effect of neurotensin, TRH and the delta-opioid receptor antagonist ICI 174864 on alcohol-induced narcosis in rats. *Brain Res* 424: 281–289.
- Wise RA and Bozarth MA (1987) A psychomotor stimulant theory of addiction. *Psychol Rev* 94: 469–492.
- Zahm DS and Heimer L (1988) Ventral striatopallidal parts of the basal ganglia in the rat: I. Neurochemical compartmentation as reflected by the distributions of neurotensin and substance P immunoreactivity. *J Comp Neurol* 272: 516–535.
- Zahm DS and Heimer L (1990) Two transpallidal pathways originating in the rat nucleus accumbens. *J Comp Neurol* 302: 437–446.