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Diverse actions of the modulatory peptide neurotensin on central synaptic transmission

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Abstract

Neurotensin (NT) is a 13 amino acid neuropeptide that is expressed throughout the central nervous system and is implicated in the etiology of multiple diseases and disorders. Many primary investigations of NT-induced modulation of neuronal excitability at the level of the synapse have been conducted, but they have not been summarized in review form in nearly 30 years. Therefore, the goal of this review is to discuss the many actions of NT on neuronal excitability across brain regions as well as NT circuit architecture. In the basal ganglia as well as other brain nuclei NT can act through diverse intracellular signaling cascades to enhance or depress neuronal activity by modulating activity of ion channels, ionotropic and metabotropic neurotransmitter receptors, and presynaptic release of neurotransmitters. Further, NT can produce indirect effects by evoking endocannabinoid release, and recently has itself been identified as a putative retrograde messenger. In the basal ganglia the diverse actions and circuit architecture of NT signaling allow for input-specific control of reward-related behaviors.

Graphical abstract

Neurotensin-induced modulation of neuronal excitability is reviewed here, revealing diverse actions across many brain regions. In midbrain dopamine neurons, neurotensin induces plasticity at glutamatergic, GABAergic, and dopaminergic synapses, leading to complex effects on motivated behavior. The diverse signaling mechanisms and actions of this neuromodulatory peptide will require further investigation but could provide promising treatments for mood and motivation-related disorders.

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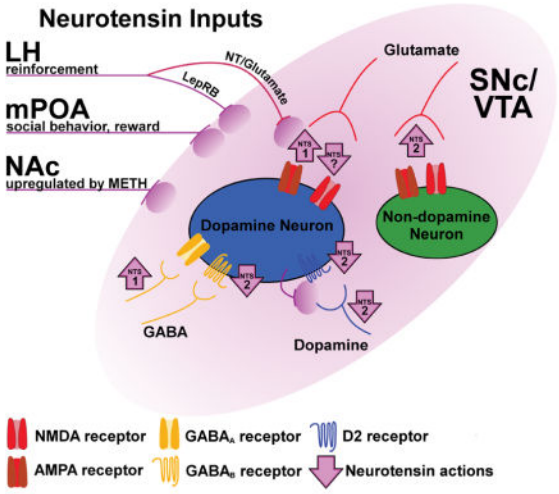

Keywords

neuropeptide; endocannabinoids; methamphetamine; VTA; substantia nigra

Introduction

Neurotensin (NT) is a signaling molecule with remarkably diverse actions. Since the initial discovery of NT by Carraway & Leeman (1973), the wide range of NT actions throughout the body has led to its study in the context of cancer, inflammation, pain, Alzheimer's disease, obesity, and anxiety (Qiu *et al.*, 2017; Black, 2002; Kleczkowska & Lipkowski, 2013; Xiao *et al.*, 2014; Li *et al.*, 2016; Shilling & Feifel, 2008, Normandeau *et al.*, 2017). The role of NT in ethanol and other drug use disorders has also been heavily studied (Erwin & Su, 1989; Erwin *et al.*, 1995, 2001; Ferraro *et al.*, 2016) and has led to an extensive accumulation of literature centered around NT action as a neuromodulator in the basal ganglia, although NT functions have been widely observed throughout the brain. Jennes and colleagues (1982) strikingly noted in their initial investigation into central NT expression that, "areas with low [NT] immunoreactivity, or lack of it, stand out." The extensive presence of NT throughout the brain is matched by its numerous reported interactions with neurons. However, there have been no recent reviews that focus on the action of NT at the level of the synapse or the expanding literature describing the role of NT circuit architecture in physiology and behavior. The current review delineates findings from rodent models regarding NT physiology including modulation of neuronal excitability and the role of NT circuitry in behavior, with emphasis on NT action in the basal ganglia and related nuclei. The diverse actions of NT, circuit-specificity of NT signaling, and modulation of a range of behaviors may allow for the development of NT-based pharmacotherapeutic treatments for mood and motivation-related disorders.

Receptor physiology

There are four known subtypes of NT receptor. The NT type-1 (NTS1) and type-2 (NTS2) receptors are G protein-coupled receptors, and the type-3 (NTS3, also known as sortilin) and type-4 (NTS4, also known as SORL1) receptors are sorting receptors, with domain structures similar to the vacuolar protein sorting 10 protein (Vps10p) domain receptor family (Tanaka *et al.*, 1990; Marcusson *et al.*, 1994; Chalon *et* al., 1996; Mazella *et al.*, 1996; Mazella *et al.*, 1998). While NT exhibits high affinity for all four receptor subtypes, NT has higher affinity at NTS1 and NTS3 (0.1–0.3 nM) compared to NTS2 (1–5 nM) and NTS4 (30 nM) (Mazella *et al.*, 1988; Jacobsen *et al.*, 2001; Pelaprat, 2006). All four NT receptors are expressed (to varying extents) in almost all brain regions (Elde *et al.*, 1990; Nicot *et al.*, 1994; Nicot *et al.*, 1995; Boudin *et al.*, 1996; Mazella *et al.*, 1996; Alexander & Leeman, 1998; Sarret *et al.*, 1998; Walker *et al.*, 1998; Motoi *et al.*, 1999; Fassio *et al.*, 2000; Sarret *et al.*, 2003a; Sarret *et al.*, 2003b; Geisler *et al.*, 2006). An exhaustive description of the precise location and expression levels of NT and NT receptors is beyond the scope of this review, but has been provided previously (Geisler *et al.*, 2006). NT receptor function proceeds through several different intracellular signaling pathways and varies by the specific cell-type on which the receptors are expressed. NTS1 has been reported to couple to $G\alpha_{i/o}$, $G\alpha_{s}$, and $G\alpha_{q/11}$ G proteins (Shi & Bunney, 1992b; Jiang et al., 1994; Wu & Wang, 1995; Gailly *et al.*, 2000; Pelaprat, 2006). The intracellular signaling mechanisms of NTS2 are not as well understood and may differ between human and mouse, but potentially involve $G\alpha_{i/o}$, $G\alpha_{q/11}$, and/or $G\alpha_{12/13}$ (Vita *et al.*, 1998; Holst *et al.*, 2004; Mazella & Vincent, 2006). There are currently no pharmacological tools available to isolate NTS3 or NTS4 activity, and as such little is known of their specific physiological function in the context of neuromodulation. Regardless, the diversity of receptors and second messenger cascades activated by NT contributes to the modulation of neuronal excitability across many cell types and brain regions.

Direct effects on neuronal excitability

NT-induced depolarization of neurons has been extensively reported in the basal ganglia and elsewhere, and usually occurs via NTS1. This has been observed in the diagonal band of Broca (Jassar *et al.*, 1999), dorsal raphe nucleus (Jolas & Aghajanian, 1996), entorhinal cortex (Xiao *et al.*, 2014), globus pallidus (Chen *et al.*, 2004), substantia nigra pars compacta (SNc; Wu *et al.*, 1995, Wu & Wang, 1995), and the ventral tegmental area (VTA; Jiang et al., 1994) (Table 1). Other direct excitatory effects of NT have also been reported, but those experiments were carried out before a NTS1 selective antagonist was available (see Shi & Bunney 1992a for review). The specific ion channels that are responsible for NTS1-induced depolarization vary by brain region. In the entorhinal cortex, NT depolarizes pyramidal cells via inhibition of the TWIK-related K^+ (TREK)-2 channel (Xiao *et al.*, 2014). Similarly, in the dentate gyrus, NT depolarizes granule cells via inhibition of the TWIK-related acid-sensitive K^+ (TASK)-3 channel (Zhang *et al.*, 2016). In the diagonal band of Broca, NT depolarizes neurons by decreasing a K^+ conductance through voltage-sensitive Ca^{2+}-activated K^+ channels, in part by modulating Ca^{2+} entry through T- and/or N-type Ca^{2+} channels (Jassar *et al.*, 1999). NT-induced depolarization of dopamine neurons in the SNc proceeds through $G\alpha_{q/11}$ activation, increasing intracellular Ca^{2+} release via inositol

triphosphate (IP3) receptors (Wu *et al.*, 1995). Single-channel analysis of this unidentified cation channel indicates that it is capable of opening in bursts and exhibits permeability to both Na^+ and Cs^+ (Chien *et al.*, 1996). It is possible that transient receptor potential (TRP) non-selective cation channels also play a role in NT effects on dopamine neurons, as application of Zn^{2+} or SKF96365 (both capable of blocking TRP channels) partially block NT-induced excitation (St-Gelais *et al.*, 2004; Stuhrman & Roseberry, 2015). Thus, NT-induced neuronal depolarization by NTS1 proceeds through different intracellular signaling cascades and ion channels depending on the cell type and brain region.

NT also increases neuronal excitability through non-NTS1 receptors. In the periaqueductal gray and nucleus raphe magnus, like in the SNc and VTA, NT directly depolarizes neurons in a $G\alpha_{q/11}$- and IP3-dependent manner (Li *et al.*, 2001a, Li *et al.*, 2001b). However, while direct depolarization is mediated by NTS1 in the SNc and VTA, in the periaqueductal gray and nucleus raphe magnus it is mediated by neither NTS1 or NTS2, suggesting a role for NTS3 and/or NTS4 (Li *et al.,* 2001a, Li *et al.,* 2001b). Similarly, neurons of the suprachiasmatic nucleus increase firing after NT application, and this effect persists in the presence of a NTS1 or non-selective NTS1/2 antagonist (Coogan *et al.*, 2001). Reports also indicate inter-species differences in the NT-induced depolarization of dopamine neurons. For instance, NT depolarizes dopamine neurons in guinea pig midbrain in two distinct ways: a slow NTS1-mediated decrease in the afterhyperpolarization observed following an action potential, and a more rapid NTS2-mediated effect (Nalivaiko *et al.*, 1998) that has not been reported in other species. Overall, NT can directly increase neuronal excitability through NTS1, NTS2, and likely NTS3 or NTS4 receptors.

Postsynaptic modulation of neurotransmitter signaling

In addition to directly depolarizing neurons, NT can also affect neuronal excitability by modulating signaling through other neurotransmitter systems. Most reports of this kind have described effects in the SNc and VTA, where NT remarkably modulates glutamate-, GABA-, and dopamine-mediated neurotransmission (Fig. 1). In the VTA, NT increases glutamatergic excitatory postsynaptic current (EPSC) amplitudes in dopamine neurons via NTS1 and non-dopamine neurons via NTS2 (Bose et al., 2015, Fig. 1). NT is also implicated in multiple extended forms of synaptic plasticity. In VTA dopamine neurons, low concentrations of NT induce long-term potentiation of NMDA receptor-mediated EPSCs through activation of NTS1 (Kempadoo *et al.,* 2013, Fig. 1). At higher concentrations, NT induces long-term *depression* of both NMDA and AMPA receptor-mediated EPSCs, and these effects are not blocked by a NTS1 antagonist (Kempadoo *et al.*, 2013, Fig. 1). Conversely, in basolateral amygdala pyramidal neurons, activation of NTS1 prevents a tetanus-induced long-term potentiation of AMPA receptor-mediated EPSCs. NT also decreases the amplitude of glutamatergic EPSCs postsynaptically in neurons of the superficial laminae of the superior colliculus (Zhang *et al.*, 1996). Thus, NT may induce or prevent long-term potentiation of glutamate neurotransmission depending on the brain region, and even within a single cell type can variously enhance or depress glutamate signaling.

In addition to glutamatergic effects, NT can also increase neuronal excitability of SNc and VTA dopamine neurons by decreasing D2 dopamine autoreceptor-mediated

neurotransmission. D2 autoreceptors are G protein-coupled receptors located on dendrites, cell bodies, and terminals of mesencephalic dopamine neurons (Sesack *et al.*, 1994; Robinson *et al.*, 2017). In the SNc and VTA, these receptors are physiologically activated via dendro-dendritic synapses between dopamine neurons (Wilson *et al.*, 1977; Groves & Linder, 1983), resulting in a powerful hyperpolarization through G protein-coupled inwardly rectifying potassium (GIRK) channels (Lacey *et al.*, 1987; Davila *et al.*, 2003; Beckstead *et al.*, 2004). NT decreases D2 autoreceptor-mediated GIRK currents in both SNc and VTA dopamine neurons by decreasing presynaptic dopamine release as well as through a postsynaptic mechanism involving protein kinase A (Shi & Bunney, 1992b; Piccart *et al.*, 2015; Stuhrman & Roseberry, 2015, Fig. 1). This NT-induced reduction of a hyperpolarizing GIRK channel conductance would be expected to increase excitability (i.e., produce disinhibition) of dopamine neurons. Unlike the IP3-dependent depolarization of dopamine neurons described above, this disinhibitory action of NT requires activation of NTS2 and persists in the absence of intracellular Ca^{2+} (Piccart *et al.,* 2015, Stuhrman & Roseberry 2015, Fig. 1). Thus, NT excites dopamine neurons in part by blunting inhibitory D2 autoreceptor signaling, both pre- and postsynaptically.

Under physiological conditions in the SNc and VTA, D2-mediated inhibition of cellular excitability proceeds predominantly through synaptic receptors, with activation of extrasynaptic receptors largely culled by dopamine transporter function (Beckstead *et al.*, 2004; Beckstead *et al.*, 2007; Ford *et al.*, 2010; Gantz *et al.*, 2013; Courtney & Ford, 2014). However, extrasynaptic D2 receptors can be readily activated when dopamine transporter-mediated uptake is inhibited by drugs such as cocaine or methamphetamine (Beckstead *et al.*, 2004; Beckstead *et al.*, 2007; Branch & Beckstead, 2012; Courtney & Ford, 2014). Action at these functionally separate populations of D2 receptors can be explored by comparing D2 signaling elicited by iontophoresis of dopamine (presumably extrasynaptic D2 signaling) with stimulation-induced release of endogenous dopamine (D2 receptor-mediated inhibitory postsynaptic currents, or D2-IPSCs; Beckstead *et al.*, 2004). Electrophysiological studies comparing currents obtained using these two methods of D2 receptor activation reveal divergent forms of regulation. For example, while low frequency electrical stimulation induces long-term depression of D2-IPSCs, currents elicited by iontophoresis of dopamine are not altered (Beckstead & Williams, 2007). NT can also induce a persistent depression of D2-IPSCs, but the effect on currents elicited by iontophoresis of dopamine is transient (Piccart *et al.,* 2015, Fig. 1). Thus, NT is apparently able to differentially regulate functional compartments of D2 receptors (presumably synaptic and extrasynaptic) on dopamine neurons. Taken together, these studies reveal diverse NT-induced modulation of dopamine signaling.

Finally, recent work shows that NT postsynaptically increases dopamine neuron excitability in the SNc and VTA by decreasing GABA signaling at $GABA_B$ receptor synapses (Stuhrman & Roseberry, 2015; Tschumi & Beckstead, 2018, Fig. 1). This form of modulation is similar to NT-induced long-term depression of D2-IPSCs in that both proceed through NTS2 activation, however NT-induced depression of $GABA_B$ signaling is transient. Furthermore, while NT-induced depression of D2-IPSCs is not dependent on protein kinase C, data from our lab indicate that $GABA_B$ depression recovers in a protein kinase C-dependent manner (Tschumi & Beckstead, 2018, Fig. 1). $GABA_B$ receptors hyperpolarize

dopamine neurons through a GIRK channel conductance that partially overlaps with that observed subsequent to activation of D2 receptors (Lacey *et al.*, 1987; Beckstead & Williams, 2007), supporting the possibility that NT decreases $GABA_B$ and D2-mediated currents at the level of GIRKs (Stuhrman & Roseberry, 2015). Overall, growing evidence indicates that NT can postsynaptically modulate neuronal signaling through a diverse array of receptors, sometimes within a single brain region.

Presynaptic modulation of neurotransmitter release

While postsynaptic effects of NT are most often excitatory, the diversity of NT actions are perhaps best illustrated in reports of presynaptic modulation of neurotransmitter release. NT increases GABA release in several brain regions. In SNc dopamine neurons, NT increases the amplitude of $GABA_A$ receptor-mediated IPSCs by increasing the readily releasable pool of GABA vesicles in a process that requires NTS1 and PKA (Tschumi & Beckstead, 2018). Interestingly, enhanced GABA release is not evident when measuring $GABA_B$ receptor-mediated IPSCs in these neurons (Tschumi & Beckstead, 2018), suggesting that presynaptic effects of NT could be localized to specific GABA inputs. In the bed nucleus of the stria terminalis, NT presynaptically induces long-term potentiation of $GABA_A$ IPSCs by increasing the probability of release of GABA (Krawczyk *et al.,* 2013; Normandeau *et al.*, 2017). In the cerebral cortex, NT hyperpolarizes pyramidal cells by increasing GABA release, however this effect *can* be observed at $GABA_B$ receptor synapses and proceeds through NTS2 (Case *et al.*, 2017). NT also increases GABA release in the hippocampal CA1 region, increasing the frequency of spontaneous IPSCs in a process that depends on NTS1, phospholipase C, IP3, and protein kinase C (Li *et al.*, 2008). In the nucleus of the solitary tract, NT exhibits the somewhat counterintuitive effect of enhancing the release of *both* glutamate and GABA, increasing the frequency of spontaneous IPSCs and EPSCs through NTS1 activation (Ogawa *et al.*, 2005). Thus, NT increases GABA release in several brain regions, but the intracellular proteins and specific alterations in synaptic signaling that mediate these effects can vary between regions.

NT also modulates the presynaptic release of glutamate and catecholamines. In the dentate gyrus, NT induces long-term potentiation of glutamate neurotransmission by increasing release probability and the number of readily releasable glutamate vesicles (Zhang *et al.*, 2015). This long-term potentiation proceeds via activation of NTS1 resulting in influx of Ca^{2+} through L-type Ca^{2+} channels, and requires activation of calmodulin and myosin light-chain kinase (Zhang *et al.*, 2015). In the parabrachial nucleus, NT increases the amplitude of evoked and spontaneous AMPA-receptor mediated EPSCs without altering currents elicited by bath application of AMPA, suggesting a presynaptic enhancement of glutamate release (Saleh *et al.*, 1997). NT also depresses cerebellar Purkinje cell firing by increasing norepinephrine release from locus coeruleus-derived afferents (Marwaha *et al.*, 1980). Finally, NT applied directly to the VTA increases dopamine neuron excitability by increasing a non-selective cation conductance and decreasing D2 autoreceptor-mediated inhibition, ultimately *increasing* DA release from dopamine neuron terminals in the nucleus accumbens (Kalivas & Duffy, 1990; Shi & Bunney, 1992a; Fawaz *et al.*, 2009). Conversely, in the SNc and VTA, and using a stimulation protocol that is too rapid to engage presynaptic autoreceptors, NT modestly *decreases* somatodendritic dopamine release at dendro-dendritic

D2 synapses (Piccart *et al.,* 2015). Altogether, depending on the brain region, NT can alter the release of glutamate, GABA, norepinephrine, or dopamine (Table 1).

Effects on endocannabinoids

NT also modulates neuronal excitability, in the basal ganglia and elsewhere, through the release of endocannabinoids (Fig. 2). In medium spiny projection neurons of the dorsolateral striatum, NT induces endocannabinoid release in a process that depends on modulation of D2 and group I metabotropic glutamate (either type-1 or type-5) receptors, with endocannabinoid activity ultimately causing long-term depression of glutamate neurotransmission (Yin *et al.*, 2008, Fig. 2). In the periaqueductal gray, NT decreases GABA neurotransmission through a chain of events: increased glutamate release activates the metabotropic glutamate type-5 receptor (mGluR5), causing the release of endocannabinoids and ultimately decreasing $GABA_A$ IPSC amplitudes (Mitchell *et al.*, 2009, Fig. 2). In midbrain dopamine neurons, NT initiates the release of the endocannabinoid 2-arachidonoylglycerol (2-AG) through NTS1-mediated activation of phospholipase C (Kortleven *et al.*, 2012, Fig. 2) which can modulate neuronal activity in two distinct ways. 2-AG acts directly on dopamine neurons to reduce A-type K^+ current, resulting in increased firing rate (Gantz & Bean, 2017, Fig. 2). 2-AG also induces long-term depression of glutamate neurotransmission (Kortleven *et al.*, 2012, Fig. 2). These two forms of modulation are seemingly contradictory in that one would be expected to increase dopamine neuron excitability through an intrinsic conductance while the other would decrease excitability by modifying glutamate input. In this case, the net effect of NT-induced 2-AG release may not be a blunt increase or decrease in firing, but rather a shift in priority toward intrinsically-driven firing and away from external signals. Interestingly, NT also *enhances* EPSCs in VTA dopamine neurons (Kempadoo *et al.,* 2013; Bose *et al.*, 2015), but the degree to which endocannabinoid signaling plays a role in this effect is unclear. Altogether, NT induces endocannabinoid release either by acting directly at postsynaptic NT receptors or indirectly by modulating signaling at D2 or metabotropic glutamate receptors.

So far, this review has covered a wide range of neuronal signals which are modulated by NT across many different brain regions. However, the net effect of physiological NT on excitability of neurons and consequent generation of behavior is less well understood and will require further investigation to determine the roles of endogenous NT signaling.

Endogenous NT signaling

Studies of NT signaling suggest that the localization and concentration of NT dictates its net effect on neuronal excitability. NT ligands applied to a single brain region can modulate neuronal activity both pre- and postsynaptically through diverse actions, and the concentration of NT ligand often dictates which of these actions predominates. For example, in two regions of the basal ganglia (the SNc and globus pallidus), exogenously applied NT ligands increase glutamate signaling at low concentrations and postsynaptically depolarize neurons at high concentrations (Chen *et al.,* 2006*,* Chen *et al.*, 2004, Kempadoo *et al.*, 2013, Shi & Bunney, 1992a). Similarly, in the nucleus of the solitary tract, low concentrations of NT increase presynaptic GABA and glutamate release, while high concentrations directly

depolarize neurons (Ogawa *et al.*, 2005). Thus, exogenously-applied NT ligands can have competing effects on neuronal excitability -- even within a single brain region -- and interpreting the physiological relevance of these effects requires a comprehensive understanding of endogenous NT signaling at the level of the synapse. Anatomical studies of peptide and receptor localization suggest that NT may signal in either a synapse-localized or more diffuse, extrasynaptic fashion. Most NT receptors in the VTA are evenly dispersed across the cell membrane with only a few receptors localized in the synapse (Dana *et al.*, 1989), suggesting that most NT signaling in this brain region occurs extrasynaptically. However, NT-containing dense-core vesicles are located in axon terminals targeting dopamine neuron perikarya (Bayer *et al.*, 1991), supporting the possibility of synapse-localized NT action.

Electrophysiological studies also suggest that NT can have synapse-localized effects. Kempadoo *et al.* (2013) demonstrated that targeted stimulation of NT-containing afferents causes NT-induced plasticity of glutamatergic signaling in the VTA. Two key experiments from this study suggest that NT is physiologically released and acts exclusively at local synapses. An important detail in the interpretation of these experiments is that exogenous NT application bi-phasically modulates glutamate signaling, with a low concentration (10 nM) enhancing and a high concentration (≥ 100 nM) depressing NMDA receptor-mediated neurotransmission. First, in recordings from a single dopamine neuron, bath perfusion of NT (10 nM) either 1) depresses EPSCs generated by stimulation of inputs to the VTA from the NT-rich lateral hypothalamus, or 2) enhances EPSCs generated by stimulation of non-specific inputs near the neuron being recorded. This suggests that localized release of endogenous NT could seemingly shift the concentration-response of exogenously-applied NT toward depression at specific synapses. The fact that depression is observed only for EPSCs from lateral hypothalamic inputs suggests that NT must act extremely locally, as other glutamatergic synapses in the same cell are not similarly affected. The second key experiment in this study uses an NTS1 antagonist to further refine the inputs involved. The NTS1 antagonist SR48692 has no effect on NMDA EPSCs elicited by stimulation of non-specific inputs, but depresses NMDA EPSCs elicited by selective stimulation of lateral hypothalamic NT-expressing inputs. Thus, NT is released and enhances signaling at only a subset of glutamatergic synapses in VTA dopamine neurons, as this effect is occluded when non-specific glutamatergic inputs are stimulated. Together, these results show that NT can modulate highly localized synaptic activity within the VTA.

There is growing evidence that NT can also act as a retrograde synaptic messenger. Krawczyk and colleagues (2013) induced NT release in the bed nucleus of the stria terminalis by applying a depolarizing step lasting 100 milliseconds and repeated at a frequency of 2 Hz for 5 minutes to the cell being recorded. This release of NT resulted in long-term potentiation of $GABA_A$ IPSCs through an increase in the probability of release of GABA. This finding is consistent with NT acting as a retrograde messenger, as long-term plasticity is induced by depolarizing the postsynaptic cell and blocked by inhibition of vesicular release within that cell (Normandeau *et al.*, 2017), but is expressed by an increase in presynaptic neurotransmitter release. Similarly, work from our lab suggests that low frequency electrical stimulation to the SNc causes NT release and long-term depression of D2-IPSCs in dopamine neurons (Piccart *et al.*, 2015). This finding is also consistent with a

putative retrograde effect of NT, as plasticity is blocked by chelating Ca^{2+} in the postsynaptic cell but expressed by a decrease in presynaptic neurotransmitter release. Anatomical evidence also supports NT action as a retrograde messenger, as NT is localized in cytoplasm and dense core vesicles in dopamine neuron dendrites in the VTA (Bayer et al., 1989). Investigations are underway to determine whether this phenomenon represents true retrograde neurotransmission or whether a local circuit is involved. Together, these studies provide fascinating new insights into the actions of endogenous NT signaling, and show that NT expression may influence both the projection targets of, and the local activity within, a variety of brain regions.

NT circuitry in behavior

The dense expression of NT neurons throughout the brain coupled with the diverse actions through which NT modulates neuronal excitability necessitate the use of circuit- and cell type-specific techniques to fully elucidate the role of NT signaling in behavior. Studies using classical techniques such as systemic and region-specific intracranial infusion of NT agonists throughout the brain, and particularly in structures of the basal ganglia, have laid the foundation for our growing understanding of NT physiology. These studies also reveal the importance of circuit-specific investigations of the role of NT signaling in behavior. One example comes from our ongoing work using an animal model of methamphetamine use disorder. In this model, mice are trained to self-administer methamphetamine through an intravenous catheter by responding operantly through a nose poke (Sharpe *et al.*, 2014; Sharpe *et al.*, 2017). We have observed that mice that are treated systemically with a NT receptor *agonist* or given an intra-VTA NT receptor *antagonist* self-administer less methamphetamine than controls (Sharpe *et al.*, 2017; Dominguez-Lopez *et al.,* 2017). Thus, NT in the VTA appears to increase, while NT in other brain regions may decrease, the reinforcing effects of methamphetamine. Conversely, methamphetamine can affect NT content in a brain region- and circuit-specific manner. For example, methamphetamine self-administration increases NT levels in the dorsal striatum, globus pallidus, nucleus accumbens shell, SNc, and VTA, but not the nucleus accumbens core or frontal cortex (Frankel *et al.*, 2011; Hanson *et al.*, 2012; Hanson *et al.*, 2013). NT mRNA is upregulated in neurons that project from the nucleus accumbens shell to the VTA (Fig. 1), but not in any of the other eleven major NT-containing inputs to the VTA (Geisler & Zahm, 2006). This suggests that there may be differences in the contribution of NT to methamphetamine use disorder not only between the VTA and other brain regions but also between individual NT inputs to the VTA. However, the contribution of specific NT-containing inputs to the VTA in the reinforcing effects of methamphetamine have not yet been explored.

Outside of studies on methamphetamine, there is evidence to suggest that NT modulates reward-related behavior in different ways depending on which inputs to the VTA are activated. Neurons that contain NT often (if not always) express other neurotransmitters, including GABA, glutamate, and dopamine (Seroogy *et al.*, 1988; Leinninger *et al.*, 2011; Kempadoo *et al.*, 2013). While the excitatory and inhibitory effects of the co-expressed neurotransmitters have not been well described, activation of NT-expressing inputs to the VTA can recapitulate the effects of intra-VTA infusions of NT agonists. For example, both intra-VTA infusion of NT and targeted (opto- or chemogenetic) stimulation of NT neurons

projecting from the lateral hypothalamus to the VTA act as reinforcing stimuli and result in increased extracellular dopamine in the nucleus accumbens (Glimcher *et al.*, 1987; Fawaz *et al.*, 2009; Kempadoo *et al.*, 2013; Patterson *et al.*, 2015, Fig. 1). Thus, activation of even a small portion of the NT-expressing inputs to the VTA is sufficient to reproduce the behavioral effects of exogenous NT application. Interestingly, activation of even smaller subsets of these neurons produces different behaviors (Fig. 1). Within the VTA, a fraction of NT-expressing neurons projecting from the lateral hypothalamus also express the receptor for the anorexigenic hormone leptin, and while both NT and leptin receptors are expressed throughout the brain, they are co-localized only in these projections (Leinninger *et al.,* 2011). Genetic ablation of leptin receptors from NT neurons results in increased feeding, altered sensitivity to amphetamine, and a decrease in the reinforcing effects of sucrose (Leinninger *et al.*, 2011; Woodworth *et al.*, 2017, Fig. 1). This suggests that NT is expressed in neuronal circuits that modulate reward-related behavior, although the degree to which changes in behavior are the result of altered NT signaling in the absence of leptin receptors is not yet clear. While the NT inputs to the VTA from the lateral hypothalamus have been characterized, a recent study suggests that other NT-inputs to the VTA also modulate reward-related behavior. Optogenetic stimulation of NT inputs from the medial preoptic area to the VTA results in increased time spent in social interaction zones and other changes in social behavior, particularly in male-female interactions (McHenry *et al.*, 2017, Fig. 1). Again, it is not clear if these changes in social behavior are due to the action of NT or other neurotransmitters co-expressed in NT inputs. Although these studies provide new insights into how NT circuit architecture modulates behavior, there is still much to learn regarding the role of specific NT inputs to the VTA as well as other brain regions. Together, these studies suggest that even a small fraction of NT afferents to a brain region can produce substantial effects on behavior.

Summary and conclusions

NT and NT receptors are highly expressed in basal ganglia nuclei and throughout the brain. NT signaling can proceed through diverse pathways and mechanisms, resulting in modulation of both intrinsic conductances and synaptic transmission in a wide range of cell types. Endogenous NT signaling likely proceeds through synapse-localized signaling and can apparently proceed in an antero- or retrograde manner. It is now clear that NT alters neuronal excitability by diverse mechanisms of action and circuit architecture that allow for input-specific control of behavior. However, circuit-specific investigations into the synaptic and behavioral effects of NT are in their infancy, and improved technology will no doubt permit ever-increasing levels of precision in future studies of this remarkable neuropeptide.

The study of NT in many different brain regions and contexts makes it difficult to assign a unitary role to this myriad modulator of synaptic activity. NT may indeed act as an analgesic in the periphery and periaqueductal gray, a neuroleptic in the striatum, a feeding peptide in the lateral hypothalamus, a socialization signal in the medial preoptic area, and an anxiogenic signal in the bed nucleus of the stria terminalis (Kleczkowska & Lipkowski, 2013; Cáceda *et al.*, 2006; Leinninger *et al.*, 2011; McHenry *et al.,* 2017; Normandeau *et al.*, 2017). However, no study has yet determined a clear action of NT under basal (non-stimulated) conditions. For example, in rodent brain slices, antagonism of NT receptors

alone almost never has any measurable effect. Only two of these studies cited in this review report any effect of NT antagonists in the absence of investigator-initiated stimulation, and those effects are minimal (Ogawa *et al.*, 2005, Coogan *et al.*, 2001). Furthermore, to our knowledge no study has reported a behavioral effect of a NT antagonist under non-stimulated conditions. It is possible that tonic NT signaling occurs at low enough levels to act in a synapse-localized fashion, modulating neuronal excitability and animal behavior at levels below the current level of detection. Perhaps more likely, the physiological role of endogenous NT in the brain may rely on phasic rather than tonic release of NT.

It is worth noting that NT signaling seems particularly well-suited to either punctuate or blunt the synaptic effects of salient stimuli in cases where fast neurotransmitter signaling is dysregulated. This statement is supported by two shared themes of NT physiology across multiple brain regions. First, NT can recruit multiple receptors and/or intracellular signaling cascades to modulate neuronal activity of single cell types. This is true in the dentate gyrus, globus pallidus, periaqueductal gray, SNc, and VTA where NT excites neurons by increasing a non-selective cation channel conductance and by either increasing glutamate or decreasing GABA signaling (See Table 1). Thus, if either ion channel expression or neurotransmitter signaling is dysregulated in a given cell, NT may yet have an excitatory effect through a backup mechanism. Second, repeated exposure to salient stimuli can upregulate NT signaling. For example, in the VTA, repeated exposure to psychostimulants results in increased expression of NT, which is itself reinforcing (Glimcher *et al.*, 1987; Geisler & Zahm, 2006; Frankel *et al.*, 2011; Hanson *et al.*, 2012; Hanson *et al.*, 2013). Also, in the bed nucleus of the stria terminalis, repeated exposure to either cocaine or unpredictable stress enhances NT-induced plasticity (Krawczyk *et al.*, 2013, Normandeau *et al.*, 2017). In this case the stimuli involved produce significant neuroadaptations, and it may be that upregulation of NT signaling is a form of homeostasis. Therefore, despite its many effects on ion channel conductances, the overall role of NT may not be to cause straightforward increases or decreases in excitability, but rather to either facilitate or combat neuronal activity or plasticity induced by salient stimuli. If a major function of NT signaling is to curb maladaptive neuronal activity, it follows that NT signaling is implicated in many disorders and highlights the importance of further investigations into NT physiology.

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List of Abbreviations

2-AG	2-arachidonoylglycerol
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
D2	dopamine D_2 (receptor)
EPSC	excitatory post-synaptic current
GABA	γ-Aminobutyric acid

$GABA_A$	GABA type-A (receptor)
$GABA_B$	GABA type-B (receptor)
GIRK	G protein-coupled inwardly-rectifying potassium (channel)
IPSC	inhibitory post-synaptic current
IP3	inositol triphosphate
METH	methamphetamine
mPOA	medial preoptic area
NMDA	*N*-methyl-D-aspartate
NT	neurotensin
NTS1	neurotensin type-1 receptor
NTS2	neurotensin type-2 receptor
NTS3	neurotensin type-3 receptor
NTS4	neurotensin type-4 receptor
SNc	substantia nigra pars compacta
TASK	TWIK-related acid-sensitive K^+ channel
TREK	TWIK-related K^+ channel TREK-2
TRP	transient receptor potential
VTA	ventral tegmental area

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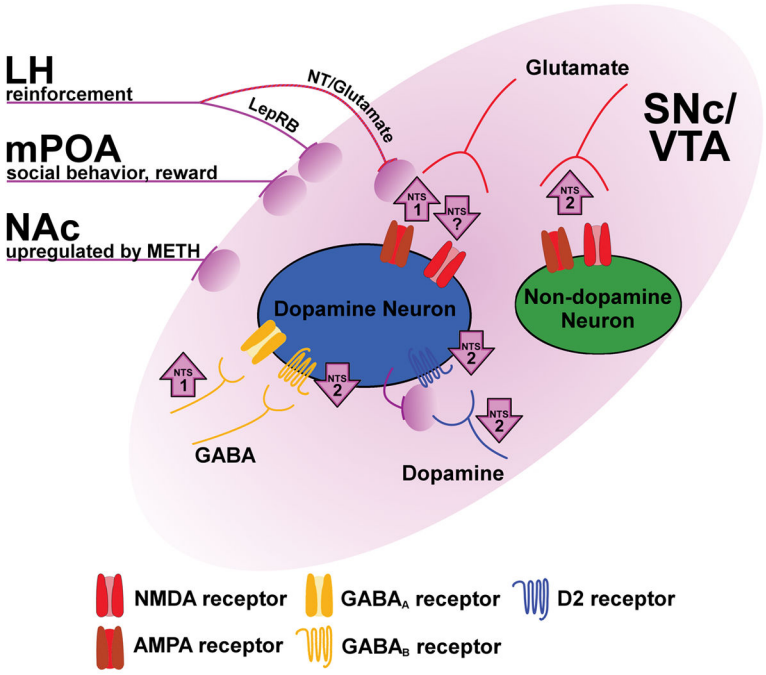

Figure 1.

Diverse NT action in the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA). This schematic indicates the architecture and behavioral roles of specific NT circuits, as well as NT-induced modulation of neurotransmitter signaling in the SNc/VTA. Purple lines and ovals represent sources of NT, including inputs from the LH that either co-express NT and glutamate (NT/glutamate) or express leptin receptors (LepRB). Purple arrows indicate an increase or decrease in signaling at a given synapse, including the responsible receptor subtype (if it is known). The location of the arrow indicates if the effect of NT occurs presynaptically (near the input), postsynaptically (adjacent to the receptor), or was not determined (between the input and receptor). NT release from dopamine neurons is putative and indicates possible retrograde transmission of NT at dendro-dendritic synapses between dopamine neurons.

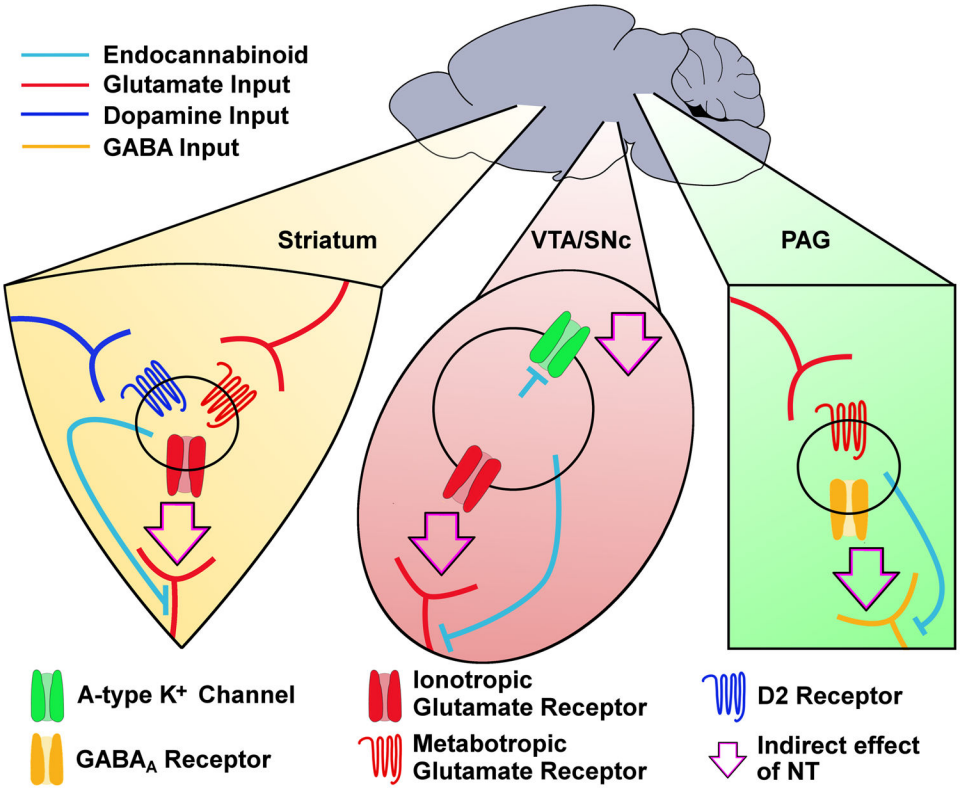

Figure 2.

NT induces endocannabinoid release in multiple brain regions. This schematic indicates actions of NT mediated by endocannabinoid release in the striatum, VTA/SNc, and periaqueductal gray (PAG). In striatal medium spiny projection neurons, NT induces endocannabinoid release in a process that depends on D2 and/or metabotropic glutamate receptor activation. Released endocannabinoids then reduce glutamatergic EPSCs. In the VTA, released endocannabinoids also reduce glutamatergic EPSCs. In SNc dopamine neurons, production of 2-AG decreases conductance through A-type K^+ channels. In the PAG, NT induces endocannabinoid release in a process that depends on metabotropic glutamate signaling. Released endocannabinoids then reduce $GABA_A$ IPSCs. Light blue lines represent paths of endocannabinoid signal. Colored lines represent glutamate (red), dopamine (dark blue), or GABA (orange) input. Arrows represent resultant endocannabinoid-induced reduction of neurotransmission or ion conductance (i.e., the indirect effect of NT receptor activation). Note that while the circles in each brain region represent postsynaptic cells in the context of endocannabinoid release, it is not clear if the D2 or metabotropic glutamate receptors necessary for NT-induced release of endocannabinoids are present on that same postsynaptic cell or if some other intermediary cell is involved.

Table 1

Neurotensin-induced modulation of neuronal excitability

Brain region	Neuron type	NT receptor(s)	Intracellular signaling	Action	Net effect	References
Amygdala (Basolateral)	Pyramidal	NTS1	--	↑ glutamate/AMPA	Excite	Amano *et al.*, 2008
Basal forebrain	Cholinergic	--	Ca^{2+}	Postsynaptic depolarization	Excite	Alonso *et al.*, 1994
Cerebral cortex	Pyramidal	NTS2	--	↑ $GABA_A$	Inhibit	Case *et al.*, 2017
			--	↑ $GABA_B$		
	Fast spiking interneuron	--	--	Depolarization	Excite	Case *et al.*, 2017
Cortex Layer 6b	Slow bursting	--	--	Postsynaptic depolarization	Excite	Combremont *et al.*, 2016
Dentate gyrus	Granule	NTS1	$G\alpha_{q/11}$	↑ TASK-3 K^+conductance	Excite	Zhang *et al.*, 2015 Zhang *et al.*, 2016
			$G\alpha_{q/11}$, calmodulin, myosin light-chain kinase	↑ glutamate/AMPA		
Diagonal band of Broca	Cholinergic	NTS1	Ca^{2+}	Depolarization	Excite	Matthews *et al.*, 1999
	Non-cholinergic	--	--	Depolarization	Excite	Jassar *et al.*, 1999
Dorsal raphe nucleus	Serotonergic	NTS1	--	Depolarization	Excite	Jolas & Aghajanian, 1996
Dorsolateral striatum	Medium spiny projection	NTS1 or NTS2	--	↑ endo-cannabinoid ↓ glutamate	Excite	Yin *et al.*, 2008
Entorhinal cortex	Pyramidal	NTS1	PLC, PKC	↓ Trek-2 K^+conductance	Excite	Xiao *et al.*, 2014
Globus pallidus	--	NTS1	PLC	↑ glutamate	Excite	Chen *et al.*, 2006 Chen *et al.*, 2004
			--	↑ non-selective cation channel conductance		
			--	↓ K^+ conductance		
Hippocampus CA1	Pyramidal	NTS1	PLC, PKC, IP3, Ca^{2+}	↑ $GABA_A$	Inhibit	Li *et al.*, 2008
	Interneuron	NTS1	--	Depolarization	Excite	
Hypothalamus	Orexin expressing	NTS1 or NTS2	--	↑ non-selective cation channel conductance	Excite	Furutani *et al.*, 2013

Brain region	Neuron type	NT receptor(s)	Intracellular signaling	Action	Net effect	References
Nucleus of the solitary tract	--	NTS1	--	↓ K^+ conductance	Excite or inhibit	Ogawa *et al.*, 2005
			--	↑ $GABA_A$		
			--	↑ AMPA		
Nucleus raphe magnus	Serotonin	NTS3 or NTS4	$G\alpha_{q/11}$, IP3, Ca^{2+}	↑ non-selective cation channel conductance	Excite	Li *et al.*, 2001b
Bed nucleus of the stria terminalis	--	NTS1 or NTS2	--	↑ $GABA_A$	Inhibit	Krawczyk *et al.*, 2013 Normandeau *et al.*, 2017
Parabrachial nucleus	--	NTS1	--	↑ glutamate/AMPA	Excite	Saleh *et al.*, 1997
Periaqueductal gray	--	NTS1	--	Depolarization	Excite	Mitchel *et al.*, 2009 Li *et al.*, 2001a
			--	↑ glutamate/mGlur5		
			--	↑ endo-cannabinoid		
			--	↓ $GABA_A$		
		NTS3 or NTS4	$G\alpha_{q/11}$, IP3, Ca^{2+}	Depolarization		
Substantia nigra pars compacta	Dopamine	NTS1	$G\alpha_{q/11}$, IP3, Ca^{2+}	↑ non-selective cation channel conductance	Excite or inhibit	Shi & Bunney, 1992b Wu *et al.*, 1995 Wu & Wang, 1995 Piccart *et al.*, 2015 Gantz & Bean, 2017 Tschumi & Beckstead, 2018
			PKC	↓ K^+ conductance		
			PKA	↑ $GABA_A$		
		NTS2	--	↓ $GABA_B$		
			--	↓ D2		
		--	PKA	↓ D2		
			--	↑ 2-arachidonyl-glycerol		
			--	↓ A-type K^+ conductance		
Supra-chiasmatic nucleus	--	NTS3 or NTS4	--	--	Excite or inhibit	Coogan *et al.*, 2001
Superior colliculus	--	--	--	↓ glutamate sensitivity	Inhibit	Zhang *et al.*, 1996
Ventral tegmental area	Dopamine	NTS1	$G\alpha_{q/11}$, PLC	↑ non-selective cation channel conductance	Excite or inhibit	Shi & Bunney, 1992b Jiang *et al.*, 1994 Chien *et al.*, 1996 Kortleven *et al.*, 2012 Kempadoo *et al.*, 2013
			PLC	↑ 2-arachidonyl-glycerol		
			--	↓ glutamate		

Brain region	Neuron type	NT receptor(s)	Intracellular signaling	Action	Net effect	References
			--	↑ glutamate/NMDA		Bose et al., 2015 Piccart *et al.*, 2015 Stuhrman & Roseberry, 2015
		NTS2 or NTS3 or NTS4	Ca^{2+}	↓ glutamate/NMDA/AMPA		
		--	--	↓ $GABA_B$		
			PKA	↓ D2		
	Non-dopamine	NTS2	--	↑ glutamate		