Neuropeptides xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Neuropeptides

journal homepage: www.elsevier.com/locate/npep

Determination of neurotensin projections to the ventral tegmental area in mice

Hillary L. Woodworth[a], Juliette A. Brown[b], Hannah M. Batchelor[a], Raluca Bugescu[a], Gina M. Leinninger[a,*]

[a] Department of Physiology, Michigan State University, East Lansing, MI, USA
[b] Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI, USA

ARTICLE INFO

Keywords:
Lateral hypothalamic area
Preoptic area
Nucleus accumbens
Retrograde tracing
Fluoro-Gold
Motivation
Reward
Dopamine

ABSTRACT

Pharmacologic treatment with the neuropeptide neurotensin (Nts) modifies motivated behaviors such as feeding, locomotor activity, and reproduction. Dopamine (DA) neurons of the ventral tegmental area (VTA) control these behaviors, and Nts directly modulates the activity of DA neurons via Nts receptor-1. While Nts sources to the VTA have been described in starlings and rats, the endogenous sources of Nts to the VTA of mice remain incompletely understood, impeding determination of which Nts circuits orchestrate specific behaviors in this model. To overcome this obstacle we injected the retrograde tracer Fluoro-Gold into the VTA of mice that express GFP in Nts neurons. Identification of GFP-Nts cells that accumulate Fluoro-Gold revealed the Nts afferents to the VTA in mice. Similar to rats, most Nts afferents to the VTA of mice arise from the medial and lateral preoptic areas (POA) and the lateral hypothalamic area (LHA), brain regions that are critical for coordination of feeding and reproduction. Additionally, the VTA receives dense input from Nts neurons in the nucleus accumbens shell (NAsh) of mice, and minor Nts projections from the amygdala and periaqueductal gray area. Collectively, our data reveal multiple populations of Nts neurons that provide direct afferents to the VTA and which may regulate specific aspects of motivated behavior. This work lays the foundation for understanding endogenous Nts actions in the VTA, and how circuit-specific Nts modulation may be useful to correct motivational and affective deficits in neuropsychiatric disease.

1. Introduction

Neurotensin (Nts) is a 13 amino-acid neuropeptide that was first extracted from the bovine hypothalamus (Carraway and Leeman, 1973). Nts has subsequently been identified throughout the brain (Beck et al., 1989; Carraway and Leeman, 1976; Jennes et al., 1982; Kitabgi et al., 1990; Uhl et al., 1979) and has been implicated in regulating a diverse repertoire of physiology and motivated behaviors including feeding, locomotor activity, social behavior, analgesia, sleep, and response to addictive drugs (Boules et al., 2011; Brown et al., 2017; Cape et al., 2000; Demeule et al., 2014; Ferraro et al., 2016; Fitzpatrick et al., 2012; Gammie et al., 2009; Merullo et al., 2015b; Smith et al., 2012). Nts may direct certain behaviors via actions in the ventral tegmental area (VTA), based on findings that different types of VTA neurons orchestrate distinct goal-directed behaviors (Lammel et al., 2012; Stamatakis et al., 2013; van Zessen et al., 2012), and that a specific subset of VTA neurons expresses neurotensin receptor-1 (Woodworth et al., 2017a). The VTA is predominantly comprised of dopamine (DA) neurons that project to and release DA in the nucleus accumbens (NA), prefrontal cortex (PFC), hippocampus, or amygdala to modify goal-directed behaviors (for review see Bromberg-Martin et al., 2010; Salamone and Correa, 2012). In contrast, the caudal "tail" of the VTA that forms a continuum with the rostromedial tegmental nucleus (RMTg) is enriched with GABA-ergic neurons, which inhibit VTA DA neurons and serve as negative regulators of DA-mediated behaviors (Carr and Sesack, 2000; Margolis et al., 2012; Tan et al., 2012; van Zessen et al., 2012). Given that alterations in both VTA DA and Nts signaling have been implicated in the pathogenesis of drug addiction, depression, anxiety, schizophrenia, autism, pain processing and obesity, there is likely functional overlap of these systems (Boules et al., 2014; Caceda et al., 2012; Carey et al., 2017; Ellenbroek et al., 2016; Ferraro et al., 2016; Fitzpatrick et al., 2012; Howes et al., 2017; Kim and Mizuno, 2010; Li et al., 2016; Nestler and Carlezon Jr, 2006; Rothwell, 2016; Theoharides et al., 2016; Volkow et al., 2013). It is therefore critical to define the precise neural mechanisms by which Nts engages the VTA, to understand how it regulates such diverse physiology and

* Corresponding author at: Department of Physiology, Michigan State University, 567 Wilson Rd, BPS Bldg, Room 3183, East Lansing, MI 48824, USA.
E-mail address: leinning@msu.edu (G.M. Leinninger).

https://doi.org/10.1016/j.npep.2018.02.003
Received 23 October 2017; Received in revised form 11 January 2018; Accepted 11 February 2018

how Nts signaling becomes maladaptive in disease.

Pharmacologic Nts activates VTA DA neurons (Legault et al., 2002; Seutin et al., 1989; Sotty et al., 2000, 1998; St-Gelais et al., 2004; Werkman et al., 2000), thereby increasing DA release in the NA (Kalivas et al., 1983; Kalivas and Duffy, 1990; Sotty et al., 2000, 1998; Steinberg et al., 1995) that can modify goal directed behaviors. Indeed, intra-VTA Nts has been shown to suppress homeostatic and motivated feeding (Cador et al., 1986; Kelley et al., 1989), increase locomotor activity (Cador et al., 1986; Elliott and Nemeroff, 1986; Feifel and Reza, 1999; Kalivas et al., 1983; Kalivas and Duffy, 1990; Kalivas et al., 1981; Panayi et al., 2005; Steinberg et al., 1994) and support self-administration (Glimcher et al., 1987; Kempadoo et al., 2013; Rompre and Gratton, 1993), conditioned place preference (CPP) (Glimcher et al., 1984; Rouibi et al., 2015) and locomotor sensitization similar to addictive drugs (Elliott and Nemeroff, 1986; Kalivas and Duffy, 1990; Kalivas and Taylor, 1985; Voyer et al., 2017). Intriguingly, many of these behavioral effects are specific to the VTA because Nts administration outside of the VTA elicits different effects. For example, while Nts injection in the VTA increases locomotor activity and DA release, intra-NA or central Nts decreases locomotor activity and does not alter DA release (Elliott et al., 1986; Kalivas et al., 1983, 1984; Meisenberg and Simmons, 1985; Vadnie et al., 2014; van Wimersma Greidanus et al., 1984). Similarly, intra-VTA Nts does not alter acute locomotor response to psychostimulants (Elliott and Nemeroff, 1986), whereas ICV or intra-NA Nts reduces psychostimulant or DA-induced hyperactivity (Ervin et al., 1981; Jolicoeur et al., 1985; Nemeroff et al., 1983; Sarhan et al., 1997; Skoog et al., 1986). One notable exception to this is Nts-mediated suppression of feeding behavior, as both central and direct administration of Nts in the VTA suppress feeding in fasted and satiated animals (Cador et al., 1986; Cooke et al., 2009; Luttinger et al., 1982). Taken together, these data suggest that there must be projection-specified populations of Nts neurons, only some of which project to the VTA to coordinate motivated locomotor and reward behaviors.

It is clear that exogenous Nts impacts the mesolimbic DA system, yet the endogenous sources of Nts to the VTA have yet to be fully characterized. Furthermore, defining Nts inputs to the VTA across species is important to determine how this neuropeptide modulates DA-mediated behaviors relevant to animal survival. For example, European starlings exhibit Nts immunoreactivity within the VTA, at least some of which originates from the preoptic area (POA); this starling Nts POA → VTA circuit is implicated in regulating sexually-motivated singing behavior (Merullo et al., 2015a, b). Zahm and colleagues have characterized endogenous sources of Nts to the VTA in rats (Geisler and Zahm, 2006a,b; Zahm et al., 2001). Abundant Nts-immunoreactive terminals are found within the VTA of rats (Beaudet and Woulfe, 1992; Hokfelt et al., 1984; Szigethy and Beaudet, 1989; Woulfe and Beaudet, 1989) and in mice, where they are found in close opposition to VTA DA neurons (Opland et al., 2013), indicating that some Nts is released to the VTA. Nts afferents to the VTA were examined in rats by injecting the retrograde tracers Fluoro-Gold (FG) and the cholera toxin beta subunit (CTb) into the VTA and using in situ hybridization (ISH) to label Nts cell bodies, demonstrating that most Nts inputs to the VTA originate from the preoptic area (POA) and rostral lateral hypothalamic area (LHA) (Zahm et al., 2001). Yet, lesioning the POA and LHA in rats does not substantially reduce Nts terminals in the VTA, suggesting there may be other important sources of endogenous input in this species (Geisler and Zahm, 2006b). Indeed, injection of a wheat germ agglutinin transynaptic tracer (WGA-apoHRP-gold) into the VTA of rats confirmed afferents from the POA and LHA, and identified putative afferents from the NA shell (NAsh), dorsal raphe (DR), ventral endoprifirom area, lateral septum (LS), pedunculopontine tegmental nucleus (PTg), and laterodorsal tegmental nucleus (LDTg) (Geisler and Zahm, 2006a).

In comparison with starlings and rats, the Nts system in mice has been comparatively little studied, and so the sources of endogenous mouse Nts input to the VTA remain incompletely understood. Studies across species were limited by the inability to easily identify Nts-expressing cells, but the recent development of *Nts*^{Cre} mice enables the facile detection and manipulation of mouse Nts neurons using Cre-Lox technology. Using these mice we have identified a large population of Nts neurons in the LHA that project to the VTA, consistent with the prior afferent mapping done in rats, and manipulation of these LHA Nts neurons reveals their crucial contributions to motivated behavior and energy balance (Brown et al., 2017; Leinninger et al., 2011; Opland et al., 2013; Patterson et al., 2015; Woodworth et al., 2017b). The LHA may be just one of several sites by which Nts orchestrates distinct behavioral responses in the mouse VTA, thus it will be important to define all Nts afferents to the VTA and test their roles individually. Indeed, *Nts*^{Cre} mice were recently used to define how medial preoptic area Nts neurons projecting to the VTA mediate social reward behavior, but this projection does not modify feeding (McHenry et al., 2017). Since these data suggest that distinct sources of mouse Nts coordinate specific DA-mediated behaviors relevant to survival (e.g. feeding vs. social behavior/mating), it is imperative to define and then to systematically test specific Nts → VTA circuits to understand their contributions to physiology and behavior. The prior literature characterizing the rat Nts system has served as a guide for mouse studies, but mice and rats differ in Nts expression (Smits et al., 2004), so they may also differ in the distribution of Nts afferents to the VTA. Thus, herein we defined the mouse Nts neurons that project to the VTA by injecting the retrograde tracer FG into the VTA of *Nts*^{Cre}*;GFP* reporter mice, permitting robust, simultaneous detection of Nts neurons and VTA afferents.

2. Materials and methods

2.1. Animals

Mice were bred and housed in a 12 h light/12 h dark cycle and cared for by Campus Animal Resources (CAR) at Michigan State University. Animals had ad lib access to chow (Teklad 7913) and water. All animal protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at Michigan State University, in accordance with Association for Assessment and Accreditation of Laboratory Animal Care and National Institutes of Health guidelines.

Nts^{Cre} mice (Leinninger et al., 2011) [Jackson stock # 017525], were bred to wild-type C57/Bl6 mice for seven generations to obtain fully backcrossed animals. To visualize Nts neurons, heterozygous *Nts*^{Cre} mice were crossed with homozygous *Rosa26*$^{EGFP\text{-}L10a}$ mice (Krashes et al., 2014), and progeny heterozygous for both *Nts*^{Cre} and *Rosa26*$^{EGFP\text{-}L10a}$ alleles were studied (referred to as *Nts*^{Cre}*;GFP* mice). Genotyping was performed using standard PCR using the following primer sequences: *Nts*^{Cre}: common forward: 5′ ATA GGC TGC TGA ACC AGG AA, cre reverse: 5′ CCA AAA GAC GGC AAT ATG GT and WT reverse: 5′ CAA TCA CAA TCA CAG GTC AAG AA. *Rosa26*$^{EGFP\text{-}L10a}$: mutant forward: 5′ TCT ACA AAT GTG GTA GAT CCA GGC, WT forward: 5′ GAG GGG AGT GTT GCA ATA CC and common reverse: 5′ CAG ATG ACT ACC TAT CCT CCC. Adult male and female *Nts*^{Cre}*;GFP* mice (ages 15–26 wk) were used for all studies.

2.2. Colchicine treatment

Stereotaxic surgeries were performed as described previously (Brown et al., 2017). Briefly, adult *Nts*^{Cre}*;GFP* mice received a pre-surgical injection of carprofen (5 mg/kg *s.c.*) and were anesthetized with 3–4% isoflurane/O_2 in an induction chamber before being placed into a mouse stereotaxic frame (Kopf). Under 1–2% inhaled isoflurane, an access hole was drilled in the skull and a guide cannula with stylet extending 0.5 mm below the cannula (PlasticsOne) was lowered into the right lateral ventricle, in accordance with the mouse brain atlas (Keith and Franklin, 2007) (A/P: −0.30 mm, M/L: −1.00 mm, D/V: −2.1 mm from Bregma). The stylet was then removed from the guide cannula and replaced with an injector that extended 0.5 mm beyond the end of the cannula. The injector was fitted with tubing attached to a