Journal of Chemical Neuroanatomy 73 (2016) 33–42

Contents lists available at ScienceDirect

Journal of Chemical Neuroanatomy

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

Multiplexed neurochemical signaling by neurons of the ventral tegmental area

David J. Barker, David H. Root, Shiliang Zhang, Marisela Morales*

Neuronal Networks Section, Integrative Neuroscience Research Branch, National Institute on Drug Abuse, 251 Bayview Blvd Suite 200, Baltimore, MD 21224, United States

ARTICLE INFO

Article history:
Received 26 June 2015
Received in revised form 31 December 2015
Accepted 31 December 2015
Available online 4 January 2016

Keywords:
Reward
Addiction
Depression
Aversion
Co-transmission
Dopamine
Glutamate
GABA

ABSTRACT

The ventral tegmental area (VTA) is an evolutionarily conserved structure that has roles in reward-seeking, safety-seeking, learning, motivation, and neuropsychiatric disorders such as addiction and depression. The involvement of the VTA in these various behaviors and disorders is paralleled by its diverse signaling mechanisms. Here we review recent advances in our understanding of neuronal diversity in the VTA with a focus on cell phenotypes that participate in 'multiplexed' neurotransmission involving distinct signaling mechanisms. First, we describe the cellular diversity within the VTA, including neurons capable of transmitting dopamine, glutamate or GABA as well as neurons capable of multiplexing combinations of these neurotransmitters. Next, we describe the complex synaptic architecture used by VTA neurons in order to accommodate the transmission of multiple transmitters. We specifically cover recent findings showing that VTA multiplexed neurotransmission may be mediated by either the segregation of dopamine and glutamate into distinct microdomains within a single axon or by the integration of glutamate and GABA into a single axon terminal. In addition, we discuss our current understanding of the functional role that these multiplexed signaling pathways have in the lateral habenula and the nucleus accumbens. Finally, we consider the putative roles of VTA multiplexed neurotransmission in synaptic plasticity and discuss how changes in VTA multiplexed neurons may relate to various psychopathologies including drug addiction and depression.

Published by Elsevier B.V.

1. Introduction

Midbrain dopamine neurons (DA) are most often associated with reward processing of both natural rewards (*e.g.*, food, water, *etc.*) and drugs of abuse (Schultz, 2002; Wise, 2004; Sulzer, 2011). Over fifty years of intense research has led to the proposal that neurons belonging to the ventral tegmental area (VTA), which includes but is not limited to DA neurons, are paramount to reward processing. Many hypotheses have been put forward regarding the specific function of VTA DA neurons in reward processing, such as decision making (Salamone and Correa, 2002a, 2002b; Saddoris et al., 2015), flexible approach behaviors (Nicola, 2010), incentive salience (Berridge and Robinton, 1998; Berridge, 2007), and learning or the facilitation of memory formation (Adcock et al., 2006; Steinberg et al., 2013). However, several studies have also shown that VTA DA neurons are involved in the processing of aversive outcomes (Laviolette et al., 2002; Young, 2004; Pezze and Feldon, 2004; Brischoux et al., 2009; Lammel et al., 2012; Twining et al., 2014; Hennigan et al., 2015), fear (Abraham et al., 2014), aggression (Yu et al., 2014a, 2014b), depression (Tidey and Miczek, 1996; Tye et al., 2013), and drug withdrawal (Grieder et al., 2014). Other hypotheses have proposed that VTA DA neurons play a more general role in processes such as associative learning (Brown et al., 2012), arousal (Horvitz, 2000), or general motivational salience and cognition (Bromberg-Martin et al., 2010).

The functional diversity associated with the VTA may be mediated, in part, by different VTA subpopulations of neurons. A particular advancement that may subserve the functional diversity of the VTA is the recent discovery of neurons that are capable of signaling using one or more neurotransmitters. In the present review, we cover recent literature on the diversity of VTA neuronal phenotypes as they relate to 'multiplexed neurotransmission'. We refer the reader to recent comprehensive reviews detailing VTA cellular composition, VTA efferent and afferents, and VTA functions (Oades and Halliday, 1987; Fields et al., 2007; Ikemoto, 2007; Nair-Roberts et al., 2008; Morales and Pickel, 2012; Trudeauet al., 2013; Morales and Root 2014; Pignatelli and Bonci, 2015; Saunders et al., 2015b; Lüthi and Lüscher, 2014). Moreover, the present review does not cover co-transmission of

* Corresponding author.
E-mail address: mmorales@intra.nida.nih.gov (M. Morales).

http://dx.doi.org/10.1016/j.jchemneu.2015.12.016
0891-0618/Published by Elsevier B.V.

neurotransmitters and neuropeptides, which has long been known and recently reviewed (Morales and Pickel, 2012). Here, we use the phrase "multiplexed neurotransmission" to describe neurons that are capable of signaling using two or more neurotransmitters. In many circuits, our understanding of the specific mechanisms by which neurons utilize multiple neurotransmitters is limited. Thus, we have chosen the term multiplexed neurotransmission to encompass known and unknown mechanisms of co-release and co-transmission (*e.g.*, Nusbaum et al., 2001; El Mestikawy et al., 2011), while also allowing for the possibility of independent release of individual neurotransmitters either in time or space.

2. Cellular diversity in the ventral tegmental area

Following the discovery of DA as a chemical neurotransmitter in the brain (Montagu, 1957), the DAergic neurons in the "ventral tegmental area of Tsai" (Nauta, 1958) were identified by formaldehyde histofluorescence (Carlsson et al., 1962). These neurons, along with other catecholaminergic and serotonergic neurons throughout the brain were shown to comprise twelve discrete cell groups (labeled as A1–A12 groups; Dahlström and Fuxe, 1964). One feature of the A10 group, in particular, is the heterogeneous morphology among its neurons. Based on cytoarchitecture, the A10 region has been divided into two lateral nuclei [the Parabrachial Pigmented Nucleus (PBP) and Paranigral Nucleus (PN)], and three midline nuclei [the Rostral Linear Nucleus of the Raphe (RLi), Interfasicular Nucleus (IF), and Caudal Linear Nucleus (CLi)]. Traditionally, the VTA has been considered to include just the lateral nuclei (PBP, PN) (Swanson, 1982), however, modern conceptions of VTA function have often included the midline nuclei (RLi, IF, CLi) as subnuclei of the VTA (Ikemoto, 2007; Nair-Roberts et al., 2008; Morales and Root, 2014). Thus, in this review, we use the term VTA to define the midbrain A10 structure containing lateral (PBP, PN) and midline nuclei (RLi, IF, CLi). The cellular heterogeneity within the VTA subnuclei, together with findings showing that a single A10 neuron rarely innervates multiple structures (Swanson, 1982; Takada and Hattori, 1987; Lammel et al., 2008; Hosp et al., 2015), suggests that the VTA utilizes highly specific projections from different sets of neurons.

Dopamine neurons, defined by the expression of tyrosine hydroxylase (TH) protein (Fig. 1), are interspersed throughout all VTA nuclei, but are most prevalent in the lateral PBP and PN (Swanson, 1982; Ikemoto, 2007; Li et al., 2013). In addition to the co-expression of TH and aromatic decarboxylase (AADC), the majority of rat lateral PBP and lateral PN neurons co-express the dopamine transporter (DAT), D2 receptor (D2R), and vesicular monoamine transporter 2 (VMAT2) mRNA (Li et al., 2013). More medially within the rat PBP and PN, as well as within the RLi, CLi, and IF, subsets of TH-expressing neurons either express or lack different combinations of DAT, VMAT2, or D2 receptor (Li et al., 2013; reviewed in Morales and Root, 2014). Our understanding of diversity among DAergic neurons in other species than the rat is less understood. However, recent studies have shown that, while all VTA neurons in the rat VTA expressing TH mRNA co-express the TH protein, some mouse VTA neurons expressing TH mRNA lack TH protein (Yamaguchi et al., 2015). In addition, ventrally to the VTA within the interpeduncular nucleus, there is in the mouse, but not in the rat, a subpopulation of neurons expressing TH mRNA, but lacking TH protein (Yamaguchi et al., 2015; Lammel et al., 2015). So far, detailed molecular characterizations of VTA neurons in nonhuman primates or humans has not been reported.

Rat TH-expressing neurons within the lateral PBP and lateral PN have also been electrophysiologically characterized (so-called 'primary' neurons) based on their long-duration action potentials and hyperpolarization-activated cation currents (Grace and Onn, 1989). However, recent findings have shown that not all VTA TH-expressing neurons share these electrophysiological criteria (Margolis et al., 2006). In addition, although lack of direct electrophysiological responses to the μ-opioid receptor agonist DAMGO has been proposed as a property shared by VTA DAergic neurons (Johnson and North, 1992), the VTA has a subpopulation of TH-expressing neurons that are directly excited or inhibited by DAMGO (Margolis et al., 2014). So far, it seems that hyperpolarization-activated cation currents, spike duration, inhibition by D2R agonist and other electrophysiological properties are unreliable predictors for the identification of all VTA DAergic neurons (Margolis et al., 2006), further supporting the heterogeneity of VTA DAergic neurons.

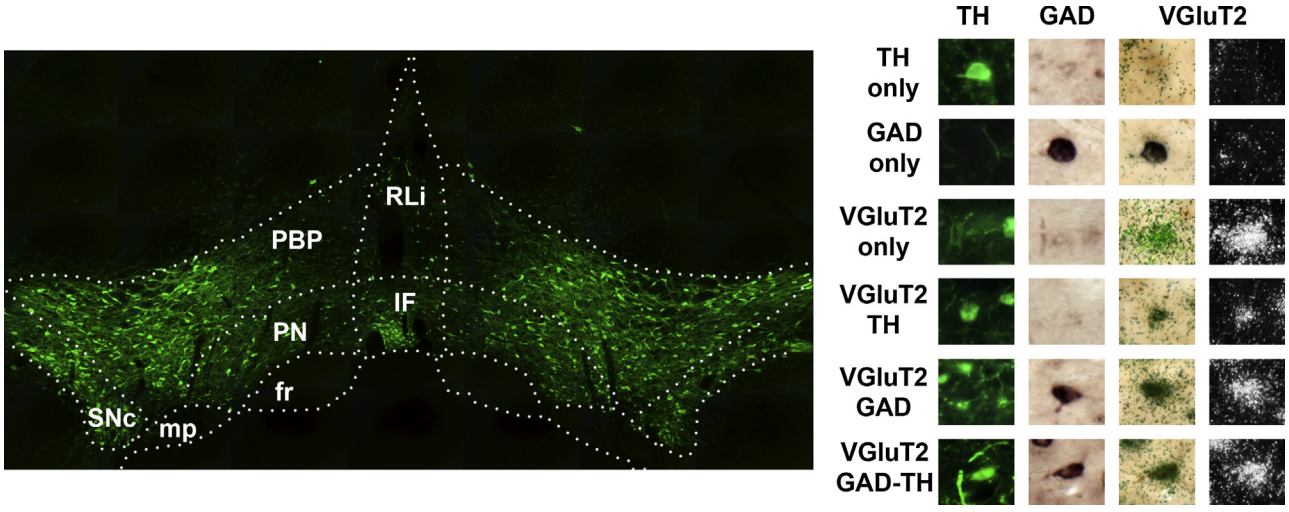

Fig. 1. Neurons in the ventral tegmental area (VTA) are capable of multiplexed neurotransmission. Detection of tyrosine hydroxylase (TH) immunoreactivity within the VTA, (low magnification, left panel). VTA combined immunohistochemistry and *in situ* hybridization showing at high magnification (right panel) neurons expressing TH (dopamine neurons; green cells), glutamic acid decarboxylase mRNA (GABA neurons; GAD 65/67; purple cells), vesicular glutamate transporter 2 mRNA (glutamate neurons; VGluT2; green or white grain aggregates) or combinations of these cell markers.
Abbreviations: Left: RLi, Rostral Linear Nucleus, IF, Interfasicular Nucleus, PBP, Parabrachial Pigmented Nucleus, PN, Paranigral Nucleus, SNc, Substantia Nigra Pars Compacta, fr, fasciculus retroflexus, mp, Mammillary Peduncle, Right: TH, tyrosine hydroxylase, GAD, glutamic acid decarboxylase, VGluT2, vesicular glutamate transporter 2.