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REVIEW

THE HETEROGENEITY OF VENTRAL TEGMENTAL AREA NEURONS: PROJECTION FUNCTIONS IN A MOOD-RELATED CONTEXT

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Abstract—The ventral tegmental area (VTA) in the brain's reward circuitry is composed of a heterogeneous population of dopamine, GABA, and glutamate neurons that play important roles in mediating mood-related functions including depression. These neurons project to different brain regions, including the nucleus accumbens (NAc), the medial prefrontal cortex (mPFC), and the amygdala. The functional understanding of these projection pathways has been improved since the extensive use of advanced techniques such as viral-mediated gene transfer, cell-type-specific neurophysiology and circuit-probing optogenetics. In this article, we will discuss the recent progress in understanding these VTA projection-specific functions, focusing on mood-related disorders.

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Key words: ventral tegmental area, projection functions, animal models, mood disorders, neural circuits, depression.

Contents

INTRODUCTION OF HETEROGENEITY OF NEURONS IN THE VENTRAL TEGMENTAL AREA (VTA)

Midbrain dopamine (DA) neurons located in the VTA have been shown to play a key role in several disorders including schizophrenia, drug addiction and mood disorders such as depression (Marinelli and White, 2000; Krishnan et al., 2007; Cao et al., 2010; Valenti et al., 2011; Chaudhury et al., 2013; Friedman et al., 2014). Classically, the VTA was thought to consist of DA neurons characterized by a slow firing rate, irregular or burst events, and a broad waveform (Yim and Mogenson, 1980; Grace and Onn, 1989). However, studies have shown that while the majority of cells in the VTA are dopaminergic (~70%), there are also small percentages of both GABA (~30%) and glutamatergic (~2–3%) neurons in this region (Yamaguchi et al., 2007; Nair-Roberts et al., 2008). Additionally, certain subpopulations of neurons have been shown to co-release two transmitters (Sulzer et al., 1998; Stuber et al., 2010; Tritsch et al., 2012). Furthermore, distinct VTA DA neurons that project to limbic regions including the nucleus accumbens (NAc), medial prefrontal cortex (mPFC), as well as the amygdala are important in mood regulation (Wise and Bozarth, 1985).

The advent of optogenetics, in addition to major advances in viral-mediated gene transfer, has allowed for the dissection of neural circuits in both a cell-type and projection-specific manner (Lobo et al., 2010; Lammel et al., 2011; Chaudhury et al., 2013; Tye et al., 2013). Here, we will focus on studies that have investigated both the functional and anatomically distinct circuits. Specifically, we will focus on studies that investigated the VTA neurons and their projections to the NAc, mPFC, and amygdala and their dysfunction in mood disorders.

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Abbreviations: AHPs, afterhyperpolarizations; BDNF, Brain-derived neurotrophic factor; BLA, basolateral amygdala; CS, conditioned stimulus; CRF, corticotropin-releasing factor; D2, DA type 2; DA, Dopamine; HCN channels, hyperpolarization-activated cyclic nucleotide-gated cation channels; LDTg, laterodorsal tegmentum; mPFC, medial prefrontal cortex; NAc, nucleus accumbens; VGLUT2, vesicular glutamate transporter 2; VTA, ventral tegmental area.

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REWARD CIRCUITRY DYNAMICS/FIRING PROPERTIES OF VTA NEURONS

Classically, midbrain DA neurons have been identified by their broad action potential waveforms and two modes of firing patterns, low-frequency tonic firing (1–5 Hz) and transient high-frequency burst or phasic firing (> 15 Hz) (Yim and Mogenson, 1980; Grace and Onn, 1989; Lammel et al., 2008; Tsai et al., 2009; Walsh et al., 2013). Further studies performed in non-human primates suggested that phasic activation of DA neurons was found to serve more in denoting the occurrence in reward-related stimuli than actually mediating the hedonic effects of reward (Schultz, 1998b). More specifically, single-unit recordings in non-human primates performing an operant task demonstrated that DA neurons could be activated by conditioned, reward-predicting stimuli (Schultz, 1998a). Occurrence of reward in the absence of a conditioned stimulus (CS) induces phasic activation of DA neurons. Further, it was seen that when a CS predicted the occurrence of reward phasic firing was elicited immediately following the CS prior to the onset of the reward. Finally, phasic activation of DA neurons occurs following a CS, however, in the failure of a reward, DA neurons are depressed at the exact expected time of the reward. Further studies in non-human primates showed that with multiple predictive stimuli phasic activation only occurred after the first predictive stimuli (Ljungberg et al., 1992). This suggests that it is the unpredictable occurrence of a reward-related stimulus that results in phasic activation.

Interestingly, studies around that time also showed that a small subpopulation of DA neurons exhibit phasic activation in response to aversive stimuli, such as air puff to the hand in non-human primates (Mirenowicz and Schultz, 1996); however, such stimuli were non-noxious. More recently, studies in C57/BL6 mice have shown that VTA DA neurons have substantial phasic activation to noxious stimuli depending on projection or neurochemical identity (Lammel et al., 2011, 2012). These studies were done using advanced viral techniques that have allowed us to more accurately parse out the different populations of VTA DA neurons.

Initially, many *in vitro* slice-recording experiments, performed both in mice and rats, suggested that VTA DA neurons were a homogenous population (Ungless et al., 2001; Argilli et al., 2008; Chen et al., 2008; Stuber et al., 2008), based on the presence of low-frequency pacemaker activity, a broad action potential, or the presence of an I_h current mediated by hyperpolarization-activated cyclic nucleotide-gated cation channels (HCN channels) (Kitai et al., 1999; Shi, 2009). In fact, some studies performed both in C57/BL6 mice and Sprague–Dawley rats, identified GABA neurons of the VTA as those lacking an I_h, thus possibly including those DA neurons now known to lack this current and skewing the interpretation of results (Ungless et al., 2001; Argilli et al., 2008). Notably, the physiological burst firing that is exhibited in VTA DA neurons is absent in *in vitro* brain slice preparations, suggesting that it is a connectivity property only seen *in vivo* (Grace and Onn, 1989). It is important to note that the criteria for identifying VTA DA neurons have generated some controversy (Ungless and Grace, 2012). Specifically, recent studies performed in both C57/BL6 and DBA/2J mice, as well as in TH-Cre rats, have shown that some VTA DA neurons have either a small or no I_h current that is dependent upon its projection region (Ford et al., 2006; Lammel et al., 2008; Zhang et al., 2010; Witten et al., 2011; Friedman et al., 2014). Other studies in albino rats of the Wistar-derived strain and in C57/BL6 mice have shown that not all VTA DA neurons undergo DA-induced inhibition (Bannon and Roth, 1983; Lammel et al., 2008). These studies suggest that VTA DA neurons are not in fact homogenous, but exhibit varying physiological characteristics (Table 1).

NEUROCHEMICAL HETEROGENEITY OF VTA NEURONS

Early *in vitro* electrophysiological studies, performed in Sprague–Dawley rats, classified DA neurons of the VTA as the primary population of neurons (Grace and Onn, 1989; Schultz, 1998a). However, later studies note that other populations of cells also exist within the VTA, GABAergic, as well as glutamatergic neurons (Nair-Roberts et al., 2008). Interestingly, studies performed in guinea-pigs show a small set of VTA neurons, some of which are dopaminergic that distinctly hyperpolarized to serotonin and opioids (Cameron et al., 1997). The neurochemical identities of all of these neurons still remain uncharacterized. GABAergic neurons within the VTA of Sprague–Dawley rats, exhibit a large amount of heterogeneity with a large range of action potential durations, firing rates, as well as both $I_h(+)$ and $I_h(-)$ cells (Margolis et al., 2012). They constitute approximately 15–20% of the entire neuronal population (Margolis et al., 2012) and synapse onto both DA and non-DA VTA neurons (Bayer and Pickel, 1991; Omelchenko and Sesack, 2009). Similar to DA VTA neurons, GABAergic VTA neurons may also play diverse roles in behavioral responses.

Some neurons in the VTA of both Sprague–Dawley rats and VGLUT1 kockout mice, express vesicular glutamate transporter 2 (VGLUT2), a marker of glutamatergic neurons, and are 2–3% of the total neuronal population, being located primarily in the rostro-medial portion of the VTA (Fremeau et al., 2004; Nair-Roberts et al., 2008). All cells contain glutamate for their role in protein synthesis, however, for exocytotic release, the VGLUTs are required (Reimer and Edwards, 2004; Takamori, 2006). Studies of cultured DA neurons express high levels of VGLUT2 (Dal Bo et al., 2004). Post-mortem brain analysis studies have used *in situ* hybridization to show that VTA DA neurons express VGLUT2 mRNA in the intact brain (Dal Bo et al., 2008; Berube-Carriere et al., 2009). Additionally, 25% of DA neurons in the midbrain of a mouse express VGLUT2 at birth as measured by single-cell RT-PCR, with this decreasing to 14% at 6-weeks of age (Mendez et al., 2008). However, the exact physiological role these neurons play remains to be elucidated.

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