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REVIEW

GLUTAMATE NEURONS WITHIN THE MIDBRAIN DOPAMINE REGIONS

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Abstract—Midbrain dopamine systems play important roles in Parkinson's disease, schizophrenia, addiction, and depression. The participation of midbrain dopamine systems in diverse clinical contexts suggests these systems are highly complex. Midbrain dopamine regions contain at least three neuronal phenotypes: dopaminergic, GABAergic, and glutamatergic. Here, we review the locations, subtypes, and functions of glutamatergic neurons within midbrain dopamine regions. Vesicular glutamate transporter 2 (VGluT2) mRNA-expressing neurons are observed within each midbrain dopamine system. Within rat retrorubral field (RRF), large populations of VGluT2 neurons are observed throughout its anteroposterior extent. Within rat substantia nigra pars compacta (SNC), VGluT2 neurons are observed centrally and caudally, and are most dense within the laterodorsal subdivision. RRF and SNC rat VGluT2 neurons lack tyrosine hydroxylase (TH), making them an entirely distinct population of neurons from dopaminergic neurons. The rat ventral tegmental area (VTA) contains the most heterogeneous populations of VGluT2 neurons. VGluT2 neurons are found in each VTA subnucleus but are most dense within the anterior midline subnuclei. Some subpopulations of rat VGluT2 neurons co-express TH or glutamic acid decarboxylase (GAD), but most of the VGluT2 neurons lack TH or GAD. Different subsets of rat VGluT2-TH neurons exist based on the presence or absence of vesicular monoamine transporter 2, dopamine transporter, or D2 dopamine receptor. Thus, the capacity by which VGluT2-TH neurons may release dopamine will differ based on their capacity to accumulate vesicular dopamine, uptake extracellular dopamine, or be autoregulated by dopamine. Rat VTA VGluT2 neurons exhibit intrinsic VTA projections and extrinsic projections to the accumbens and to the prefrontal cortex. Mouse VTA VGluT2 neurons project to accumbens shell, prefrontal cortex, ventral pallidum, amygdala, and lateral habenula. Given their molecular diversity and participation in circuits involved in addiction, we hypothesize that individual VGluT2 subpopulations of neurons play unique roles in addiction and other disorders.
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Key words: ventral tegmental area, substantia nigra, retrorubral field, dopamine, VGluT2, Addiction.

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Midbrain dopamine (DA) neurons are hypothesized to play roles in reward-based behavior and addiction (Wise, 1978, 2008), reward prediction and learning by error detection (Schultz and Dickinson, 2000), effort-based decision making (Salamone and Correa, 2002), flexible reward-directed behaviors (Ikemoto and Panksepp, 1999; Nicola, 2010), incentive salience (Berridge, 2007), stimulus salience (e.g., prediction of rewarding and aversive events; Young et al., 2005), aversion (Lammel et al., 2014; Volman et al., 2013), depression (Nestler and Carlezon, 2006; Yadid and Friedman, 2008), and fear (Pezze and Feldon, 2004). The extensive, divergent behavioral roles of midbrain dopamine neurons, predominantly from the ventral tegmental area (VTA), indicate that this system is highly heterogeneous. This heterogeneity may be reflected in part by the diverse phenotypic characteristics among DAergic neurons and their interactive brain structures (Ford et al., 2006; Margolis et al., 2006a,b, 2008; Morales and Pickel, 2012; Li et al., 2013; Ford, 2014; Lammel et al., 2014; Overton et al., 2014; Yetnikoff et al., 2014).

The midbrain DAergic neurons are interspersed with GABAergic neurons and with glutamatergic neurons (Kawano et al., 2006; Yamaguchi et al., 2007, 2011, 2013; Nair-Roberts et al., 2008). Based on electrophys-

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Abbreviations: DAT, dopamine transporter; EPSC, excitatory postsynaptic current; mPFC, medial prefrontal cortex; nAcc, nucleus accumbens; PBP, parabrachial pigmental; PN, paranigral nuclei; RRF, retrorubral field; SNC, substantia nigra pars compacta; TH, tyrosine hydroxylase; VMAT2, vesicular monoamine transporter; VGluT, vesicular glutamate transporter 2; VTA, ventral tegmental area; WT, wild type.

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iological and pharmacological properties, *ex vivo* electrophysiological recordings from midbrain neurons have provided evidence for three subpopulations of neurons (primary, secondary and tertiary neurons) (Grace and Onn, 1989; Johnson and North, 1992a,b; Cameron et al., 1997; Ungless et al., 2004). The subpopulation of primary neurons has been recognized as DAergic neurons that in their majority have long duration action potentials and hyperpolarization-activated cation current (Ih) (Grace and Onn, 1989). In contrast, the subpopulation of secondary neurons has been recognized as GABAergic neurons with short action potential durations and without Ih (Johnson and North, 1992a,b). The third subpopulation of neurons lacks the electrophysiological properties associated with DAergic or GABAergic neurons, and it has been suggested to use glutamate as a signaling molecule (Ungless et al., 2004).

The presence of glutamatergic neurons within the midbrain DA regions was initially suggested from *in vivo* (Wilson et al., 1982; Mercuri et al., 1985; Ungless et al., 2004; Lavin et al., 2005; Chuhma et al., 2009), and *in vitro* electrophysiological findings (Sulzer et al., 1998; Joyce and Rayport, 2000; Chuhma et al., 2004). *In vivo* studies have shown that electrical stimulation of the substantia nigra pars compacta (SNC) evokes excitatory postsynaptic currents (EPSCs) in dorsal striatal neurons (Wilson et al., 1982). The presence of a nigrostrial glutamatergic pathway has been further supported by ultrastructural analysis of anterograde-labeled material showing axon terminals originating from the SNC lacking tyrosine hydroxylase (TH, marker of midbrain DA neurons), and making putative excitatory asymmetric synapses in the dorsal striatum (Hattori et al., 1991). Electrical stimulation of the neighboring VTA also evokes EPSCs in neurons within the medial prefrontal cortex (mPFC, Mercuri et al., 1985; Lavin et al., 2005), and within the nucleus accumbens (nAcc, Chuhma et al., 2009).

The earliest electrophysiological *in vivo* studies reporting EPSCs evoked by midbrain electrical stimulation did not ascribe these excitatory responses to the release of glutamate from DA neurons (Wilson et al., 1982; Mercuri et al., 1985). However, later studies proposed release of glutamate from DA neurons as a mechanism to evoke EPSCs from VTA efferents (Lavin et al., 2005; Chuhma et al., 2009). The idea that glutamate is released by DAergic neurons was initially proposed by Kaneko et al., 1990 based on the observation that antibodies against glutaminase were able to immunolabel all catecholaminergic neurons. However, glutaminase is an enzyme necessary for the production of metabolic glutamate, as such is present in many non-glutamatergic neurons (Laake et al., 1999), thus glutaminase is not a selective marker for glutamate signaling neurons. Nevertheless, *in vitro* electrophysiological studies have shown glutamatergic signaling by midbrain-cultured DA neurons (Sulzer et al., 1998; Joyce and Rayport, 2000; Bourque and Trudeau, 2000; Sulzer and Rayport, 2000) and midbrain slices (Chuhma et al., 2004, 2009).

ANATOMICAL IDENTIFICATION OF MIDBRAIN GLUTAMATERGIC NEURONS

The analysis of glutamatergic neurons has been greatly advanced in the last decade due to the cloning of three distinct vesicular glutamate transporters (VGluT1, VGluT2 and VGluT3), which accumulate glutamate into vesicles for its synaptic release (Bellocchio et al., 1998; Takamori et al., 2000; Bai et al., 2001; Fremeau et al., 2001; Fujiyama et al., 2001; Hayashi et al., 2003; Herzog et al., 2001; Varoqui et al., 2002). VGluT1 and VGluT2 are restricted to known glutamatergic neurons, and their presence has become a reliable molecular marker to identify the distribution and synaptic connectivity of glutamatergic neurons within different brain regions. While VGluT1 and VGluT2 are highly concentrated in synaptic vesicles in axonal terminals of glutamatergic neurons (Fremeau et al., 2001; Fujiyama et al., 2001; Herzog et al., 2001), they are undetectable in other neuronal compartments, such as cell bodies, dendrites, and axons. Thus, cellular detection of mRNA transcripts encoding VGluT1 or VGluT2 is so far the only available and reliable method to identify cell bodies of glutamatergic neurons in non-transgenic animals. To detect glutamatergic neurons within the rat DA midbrain regions, *in situ* hybridization methods have been applied with the use of radioactive and non-radioactive probes (Kawano et al., 2006; Yamaguchi et al., 2007; 2011; 2013; Nair-Roberts et al., 2008; Bérube-Carriére et al., 2009). These *in situ* hybridization studies have shown the following major findings: first, that in the adult rat there are neurons expressing VGluT2 mRNA, but not VGluT1 nor VGluT3, in the VTA (Kawano et al., 2006; Yamaguchi et al., 2007), in the SNC (Yamaguchi et al., 2013), and in the retrorubral field (RRF; Yamaguchi et al., 2013). Second, that different experimental conditions used for detection of VGluT2 mRNA in the adult rat may explain discrepancies in the number of VGluT2-expressing neurons detected in the VTA (Kawano et al., 2006; Bérube-Carriére et al., 2009; Yamaguchi et al., 2007, 2011), SNC and RFF (Nair-Roberts et al., 2008;Yamaguchi et al., 2013). Third, that the VGluT2 neurons within the RRF, SNC and neighboring lateral aspects of the VTA [lateral aspects of parabrachial pigmental (PBP) and paranigral nuclei (PN)] appear to be similar to each other, but different from those present in the midline nuclei of the A10 region (medial aspects of both PBP and PN; rostral linear nucleus, RLi; interfascicular nucleus, IF and caudal linear nucleus, CLi). Four, that although some VGluT2 neurons in the midline nuclei of the VTA co-express TH, the vast majority of VGluT2 neurons lack TH within the mature rat RRF, SNc, and lateral VTA (Yamaguchi et al., 2011, 2013; Li et al., 2013). These four major findings will be further detailed in this review.

GLUTAMATERGIC NEURONS WITHIN THE RRF AND THE SNC

By applying radioactive *in situ* hybridization in combination with TH immunolabeling, we have found that the vast majority of VGluT2-expressing neurons do not co-express TH within the RRF, SNC (Yamaguchi

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