



Presynaptic regulation of dopamine release: Role of the DAT and VMAT2 transporters

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ABSTRACT

The signaling dynamics of the neurotransmitter dopamine has been established to have an important role in a variety of behavioural processes including motor control, cognition, and emotional processing. Key regulators of transmitter release and the signaling dynamics of dopamine are the plasma membrane reuptake transporter (DAT) and the vesicular monoamine transporter (VMAT2). These proteins serve to remove dopamine molecules from the extracellular and cytosolic space, respectively and both determine the amount of transmitter released from synaptic vesicles. This review provides an overview of how these transporter proteins are involved in molecular regulation and function together to govern the dynamics of vesicular release with opposing effects on the quantal size and extracellular concentration of dopamine. These transporter proteins are both focal points of convergence for a variety of regulatory molecular cascades as well as targets for many pharmacological agents. The ratio between these transporters is argued to be useful as a molecular marker for delineating dopamine functional subsystems that may differ in transmitter release patterns.

The role of dopamine as a signaling molecule within the central nervous system relies on the complex coordination of a multitude of physiological mechanisms acting in concert. Of these mechanisms, the current paper focuses on primarily three: the quantal size (the amount of dopamine released per single vesicle fusion event), the plasma membrane transporter, and the vesicular membrane transporter. In order to bind with post-synaptic receptors, the dopamine transmitters must be released from the pre-synaptic neuron terminal, and it is now well recognized that the amount released is subject to change based on factors affecting quantal size (Pereira and Sulzer, 2012; Schmitz et al., 2003). In essence, the release of dopamine may be seen as a transference between compartments, from the intracellular side of the plasma membrane to the extracellular side.

The compartment concept of dopamine action has been used previously in research (Grace et al., 2007). Applying this concept, the extracellular space is regarded as only one compartment of possible dopamine action. The cytoplasm within the neuron, and the intraluminal space within a secretory vesicle may be considered additional distinct intracellular compartments (Uhl, 1998). Thus, the function of dopamine may be dependent on what compartment it is in. The movement and regulation of dopamine within and between compartments is thereby critical to its function. Two transporter proteins are central in regulating transfer of dopamine between these compartments: the plasma membrane transporter (DAT) and the vesicular

membrane transporter (VMAT, German et al., 2015; Lohr et al., 2017). These transporters function in concert to regulate the activity of dopamine and have influence on each other, which alters dopamine quantal size (Pereira and Sulzer, 2012; Schmitz et al., 2003).

1. The dopamine terminal: An overview

Dopamine is one of the catecholamine neurotransmitters and is produced from the precursor tyrosine. This aromatic amino acid is converted by the rate-limiting enzyme tyrosine hydroxylase (TH) into L-3,4-dihydroxyphenylalanine (L-DOPA; Levitt et al., 1965). L-DOPA is subsequently converted to dopamine via a decarboxylation accomplished by the enzyme aromatic amino acid decarboxylase (AADC; Christenson et al., 1972). In dopamine terminals, this represents the final step of the biosynthetic pathway (see Fig. 1), where dopamine molecules may then be sequestered and packaged into secretory vesicles via transport proteins (Liu and Edwards, 1997). The activity of TH largely governs overall rate of synthesis, and this enzyme is subject to extensive regulation including from end-product inhibition (Masserano and Weiner, 1983). It should be noted that there are a variety of regulatory molecular cascades in addition to this end-product inhibition, however, these fall outside of the scope of the current review (for reviews see, Tekin et al., 2014; Zigmond et al., 1989).

Traditionally, the synthesis of dopamine is thought to occur

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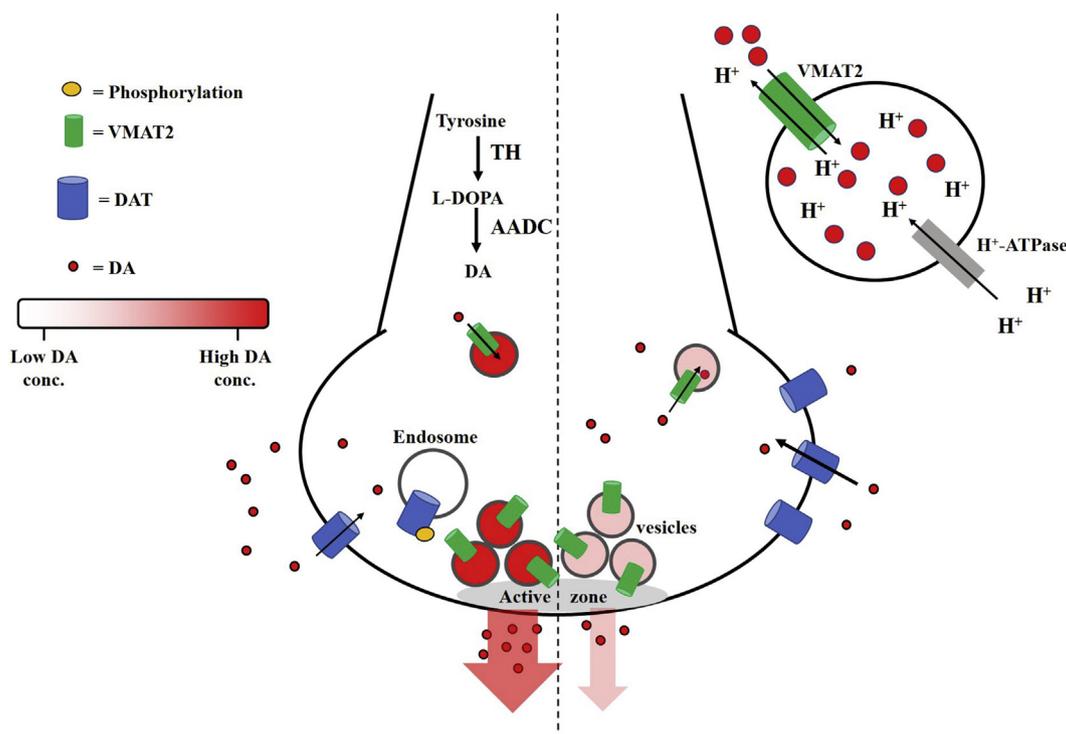


Fig. 1. Simplified model of presynaptic dopaminergic terminal illustrating relative implications of varying DAT expression levels on vesicular, cytosolic and extracellular transmitter concentrations. Left side of the diagram shows increased quantal size and increased release of dopamine molecules and the right side showed decreased quantal size and release (sides separated by dashed line). Intensity of the red colour signifies concentration of dopamine, as explained in the rectangle below the legend on the left hand side. Synthesis of dopamine (DA) indicated with lines representing conversion of tyrosine to L-DOPA via enzymatic function of tyrosine hydroxylase (TH) and conversion of L-DOPA to DA via enzymatic function of aromatic amino acid decarboxylase (AADC). Top right corner represents a magnified view of the vesicular loading process utilizing an exchange of protons for DA molecules. The gray rectangle represents a vacuolar-type H^+ -ATPase which utilizes ATP to transport protons into the vesicle. The size of the proteins and compartments are not to physiological scale.

independently from vesicular packaging, whereby it involves an intermediate cytosolic step for the dopamine molecules before they are loaded into vesicles. However, there are recent lines of evidence that suggest functional protein associations between the dopamine synthesis enzymes (TH and AADC) and formation and packaging of the secretory vesicles (Cartier et al., 2010; Chen et al., 2003; Tsudzuki and Tsujita, 2004). This association suggests that direct synthesis-packaging coupling occurs that introduces an avenue for largely bypassing end-product inhibition of TH activity from newly synthesized dopamine molecules.

The degradative and signaling termination mechanisms for dopamine transmission are primarily comprised of the enzymes monoamine oxidase B (MAO-B; located inside the presynaptic terminal), Catechol-O-methyltransferase (COMT; located inside the synaptic cleft), and transporter-mediated removal of dopamine from the synaptic cleft (German et al., 2015; Kopin, 1985).

2. The dopamine plasma membrane transporter protein: the DAT

The plasma membrane protein responsible for the reuptake of dopamine from the extracellular space (henceforth referred to as DAT) is a critical regulator of dopaminergic neurotransmission in the central nervous system (CNS) of mammals (Lohr et al., 2017; Schmitz et al., 2003; Sotnikova et al., 2006; Torres et al., 2003). Though there are degradative enzymes, this transmembrane reuptake transporter is the primary mechanism of dopamine clearance from the extracellular space (Benoit-Marand et al., 2000; Gonon et al., 2000; Jones et al., 1998). Its transport kinetics helps to shape the temporal and spatial dynamics of dopaminergic action on post-synaptic receptors (Cragg and Rice, 2004; Schönfuß et al., 2001). Thus, it has been a longstanding target of intense research.

2.1. Pharmacological manipulation of the DAT

There are a variety of pharmacological agents that can affect DAT function with profound effects on dopamine neurotransmission (Jones et al., 1999; Reith et al., 2015.) Some of these drugs are utilized in a therapeutic capacity, such as methylphenidate and modafinil for ADHD and narcolepsy, respectively (Krause et al., 2005; Volkow et al., 2009a,b). Many drugs that affect the DAT are well known drugs of abuse in humans (such as cocaine, D-amphetamine, MDMA, or methamphetamine; Giros et al., 1996; Heikkila et al., 1975; Ritz et al., 1987; Zhu and Reith, 2008). This abuse potential along with the concomitant neurotoxicity greatly complicates the practice of their clinical use as therapeutics (Ares-Santos et al., 2014; Granado et al., 2008; Howell and Negus, 2014; Volkow et al., 2009a,b).

An important categorization often employed regarding drugs that affect DAT function is that of classifying their effect as one of transporter blockade or reversal. The archetypes of these classifications are cocaine and amphetamine for transporter blockade and reversal, respectively (for reviews see, German et al., 2015; Sulzer, 2011). It should be noted that many of these drugs have pharmacological effects beyond DAT modulation. These drugs often affect general monoaminergic systems, and in certain cases (i.e. amphetamines) may affect secretory vesicles, dopamine synthesis, and activity of degradative enzymes (Sulzer, 2011; Sulzer et al., 2005). DAT blockers (cocaine, methylphenidate, etc.), inhibit the effective re-uptake of dopamine molecules from the extracellular space and thereby increase the synaptic cleft concentration of dopamine in a manner dependent on vesicle exocytosis (Jones et al., 1995a,b; Venton et al., 2006).

In contrast to blocking functional activity of the DAT protein, some drugs (e.g. D-amphetamine) act as substrates and reverse the transport activity of the protein (Fischer and Cho, 1979; Heikkila et al., 1975).

This reversal leads to DAT-mediated efflux of dopamine molecules from the intracellular side of the presynaptic terminal into the extracellular space in a manner independent of vesicle exocytosis (for more exhaustive reviews see, Fleckenstein et al., 2007; Sitte and Freissmuth, 2015). Amphetamine-induced dopamine release requires functioning DAT molecules so that amphetamine may be transported into the terminal (this may be blocked by DAT antagonists such as cocaine; Zaczek et al., 1991). From within the terminal, amphetamine may then initiate a concert of actions which lead to profound dopaminergic efflux through the DAT protein. In brief, these actions include: depletion of synaptic vesicle stores which increases cytoplasmic concentration of dopamine (Sulzer et al., 1995), reduction of degradative enzyme activity on cytoplasmic dopamine via amphetamine inhibition of MAO-B (Mantle et al., 1976), and increases in the frequency of channel-like pore formation enabling extensive non-vesicular release of transmitter (Kahlig et al., 2005). However, there is evidence that post-translational modifications of the DAT protein are necessary for the amphetamine-induced efflux of transmitter (Khoshbouei et al., 2004; Pizzo et al., 2013; 2014), which highlights the integral role of functional regulation of plasmolemmal transporters in modulating neurotransmission. For instance, DAT association with syntaxin1A, a SNARE protein associated with vesicular release, appears to promote the formation of channel-like mode of dopamine efflux induced by amphetamine (Binda et al., 2008). Interestingly, there is evidence that suggests this form of dopamine efflux inducible by amphetamine may occur endogenously and underlie human disorders associated with DAT coding variants (Bowton et al., 2010; Mazei-Robison et al., 2008).

With its integral role in regulating dopaminergic function, the DAT is an important protein for understanding a variety of topics, including addiction (Zahniser and Sorkin, 2004; Zhu and Reith, 2008), motivation (Bromberg-Martin et al., 2010), emotional expression (Brudzynski, 2007; Brudzynski et al., 2018), and motor function (Fernagut et al., 2003).

2.2. Function and kinetics of the DAT

The DAT belongs to a large family of Na⁺–Cl[–]-coupled neurotransmitter transporters (the SLC6 gene family), a family that also consists of transporters for serotonin, norepinephrine, glycine, and GABA (Chen and Reith, 2000; Chen et al., 2004; Gether et al., 2006). The similarity of transporters for the uptake of biogenic monoamines (e.g., dopamine, serotonin, and norepinephrine) is evidenced by drugs that lack selectivity among them (i.e., the tricyclic antidepressants; Waldmeier et al., 1976). However, the emergence of drugs that show some ability to selectively target and differentiate the various monoamine reuptake transporters has been critical in elucidating their specific structure and function (Gether et al., 2006; Graham and Langer, 1992). It must be mentioned that despite distinct pharmacological properties, *in vivo* observations indicate that the dopamine and norepinephrine reuptake transporters share considerable overlap in substrate specificity (Torres et al., 2003). The norepinephrine transporter (NET) was shown to be capable of uptake of dopamine molecules and may be involved in regulating extracellular dopamine in regions low in DAT (Morón et al., 2002; Yamamoto and Novotney, 1998). Despite this functional overlap, the expression of the DAT and NET appears to be restricted to their cell types only, dopaminergic and noradrenergic cells, respectively (Lorang et al., 1994). Thus, even with NET involvement, a focus on the DAT in relation to the functioning of dopaminergic cells is justified.

The transporters of the SLC6 family (thus the DAT) rely on sodium and chloride ions and the electrochemical driving force pushing them towards the inside of the cell (Chen et al., 2004; Kristensen et al., 2011). The transporters use the movement of sodium ions (two sodium ions for one neurotransmitter molecule) to co-transport the neurotransmitter in symport fashion (Kristensen et al., 2011; Shi et al., 2008). The sodium ions provide an energy force directed towards the intracellular side of

the membrane that the transporter uses to move the neurotransmitter against even a large concentration gradient (Feldman et al., 1997). Thus, the reuptake process is one of secondary active transport in that the energy required to move the molecule of dopamine is indirectly using ATP via the sodium-potassium pump maintaining the electrochemical potential of sodium ions across the neuronal membrane (Kristensen et al., 2011). Disrupting the membrane potential (e.g. by action of ouabain, an inhibitor of the Na/K-ATPase) is thus able to potentially inhibit neurotransmitter uptake (Feldman et al., 1997).

The kinetics of transport follows the Michaelis-Menten model whereby maximal uptake velocity is dependent initially on substrate concentration, whereas at high substrate concentrations, the number of active transporters dictates maximum uptake (Kristensen et al., 2011; Schmitz et al., 2003). The mechanism of transport relies on alternating access between inward and outward conformations of the protein. Upon substrate binding, while the active part of the protein is facing extracellularly, a conformational change is induced in the protein that enables it to translocate the substrates to the intracellular side (Chen et al., 2003; Kristensen et al., 2011). A secondary substrate binding reaction then occurs to induce the protein to release the translocated substrate into the cytosol (Shi et al., 2008). The functional role of the protein's conformation is important for consideration as many DAT antagonists appear to differ in their effect via stabilizing either the inward or outward facing conformation phase (Reith et al., 2015; Schmitz et al., 2013). There is also evidence that the DAT is capable of mediating channel-like movement of ions, uncoupled from dopamine reuptake, which can affect the plasma membrane potential. This putative channel function appears capable of producing depolarizing currents that may modulate functional behaviour of the neuron (Carvelli et al., 2004; Ingram et al., 2002).

Through its reuptake of dopamine from the extracellular space, the DAT is critical in regulating both the extra- and intra-cellular concentrations of dopamine. This recycling of dopamine allows for less dependence on dopamine synthesis and prolonged vesicular release (Benoit-Marand et al., 2000; Gainetdinov et al., 1998). With its function, the DAT determines the cytosolic concentration of dopamine in direct relation to the level of cellular activity causing neurotransmitter release. Greater dopamine release will induce a higher concentration of cytosolic dopamine available for vesicular packaging independent of synthesis (Chen and Reith, 2000; Lohr et al., 2017; Pereira and Sulzer, 2012). Moreover, the kinetics and function of the DAT is dynamic and subject to alteration from a variety of regulatory elements (Mortensen and Amara, 2003).

2.3. Molecular structure and regulation of the rodent DAT

Structurally, rodent DATs are 619 amino acid proteins with 12 transmembrane domains that have both N- and C-termini located within the cytosol (Chen and Reith, 2000; German et al., 2015). There are a variety of amino acid residues that are subject to post translational modification and functional regulation (e.g. glycosylation, phosphorylation, etc.) by protein kinases and other signaling molecules (Foster et al., 2002; Vaughan et al., 1997). These reactive sites are located extensively both on the intra- and extra-cellular sides of the plasma membrane. Within the cytoplasm, the N- and C-terminal tails both represent large regulatory elements that interact with a multitude of signaling molecules in an intricate fashion (Ramamoorthy et al., 2011; Vaughan and Foster, 2013).

A description of the molecular regulation of DAT function, though complex, serves to emphasize that the DAT represents an important node in many convergent and divergent pathways of intracellular physiological processes (Mortensen and Amara, 2003; Vaughan and Foster, 2013). Generally, in addition to site-specific modification, the N and C tails also interact with various binding partners (i.e. syntaxin1A, dopamine autoreceptors, etc.) (Binda et al., 2008; Lee et al., 2007). These dynamic molecular interactions serve to either facilitate or

inhibit DA transport capacity (Ramamoorthy et al., 2011; Vaughan and Foster, 2013).

One primary way transport may be inhibited is via PKC-mediated phosphorylation of the DAT protein. Protein kinase C (PKC) is an example of a signaling molecule that acts as a downstream regulatory effector of many signaling pathways (Foster et al., 2002; Vaughan et al., 1997). PKC induced inhibition of DAT may take the form of membrane trafficking that sequesters and internalizes the transporter proteins (Loder and Melikian, 2003). The PKC-mediated phosphorylation stimulates clathrin- and dynamin-mediated endocytosis, which removes the DAT from the surface of the plasma membrane (Loder and Melikian, 2003). By removing the DAT in this manner, there is a reduction of its surface expression levels and thereby a reduction of total transport capacity (even in high substrate concentration conditions; Vaughan and Foster, 2013). This endocytotic regulation may be short and acute (rapid trafficking to and from surface), or long term via lysosomal degradation (Hong and Amara, 2013). The PKC activated endocytosis is related to a wide variety of other regulatory mechanisms. For example, N-terminal ubiquitylation has been found to mediate PKC stimulated endocytosis (Miranda et al., 2007). Alternatively, without changes to plasma membrane expression levels (i.e., in an endocytosis-independent manner), the DAT protein itself may be inhibited. PKC stimulated phosphorylation was found to reduce the functional kinetics of DAT molecules independent of any trafficking process (Foster et al., 2008; Moritz et al., 2015). This mechanism of kinetic regulation represents a more rapid form of DAT inhibition and likely operates in concert with membrane trafficking (Moritz et al., 2015).

In contrast to DAT inhibition, signaling molecules may also activate an enhancement and upregulation in dopamine transport. As with inhibition, upregulation can happen both kinetically per DAT molecule, or through membrane-trafficking and alterations to the levels of DAT expressed at the surface. Regulatory modifications to the molecules (such as palmitoylation) can alter the kinetics of the DAT molecule to increase uptake in a manner that is membrane-trafficking independent (Foster and Vaughan, 2011; Rastedt et al., 2017). Moreover, this palmitoylation exerts an opposing effect on PKC-mediated phosphorylation and can prevent PKC-stimulated endocytosis (Foster and Vaughan, 2011).

This is just one example of the intricate and reciprocal regulations that are exerted on DAT molecules to alter their transport kinetics, which occur rapidly and reversibly (Moritz et al., 2015). Additionally, there are membrane trafficking-based mechanisms of transport enhancement that occur to increase the expression levels of DAT on the plasma membrane surface. For instance, direct protein-protein interactions between autoreceptors (D₂ receptors) and DAT molecules can occur. These interactions physically translocate transporter proteins to the surface to facilitate expression on the plasma membrane and thereby increase total transport capacity (Lee et al., 2007).

The extensive regulation of DAT molecules indicates that they are dynamic contributors to the regulation of dopamine activity. Under such regulatory pressures, the DAT molecules represent a tool by which many independent cellular systems may exert control over dopaminergic function (Gowrishankar et al., 2014; Mortensen and Amara, 2003; Vaughan and Foster, 2013; Zahniser and Sorkin, 2004).

2.4. DAT expression in the rodent brain

DAT expression, which is selective to dopaminergic neurons within the brain, is found extensively among the many brain regions associated with dopaminergic activity (Ciliax et al., 1995; Freed et al., 1995; Lorang et al., 1994). This includes the nine dopamine cell groups (A8–A16) denoted by the taxonomy of Dahlström and Fuxe (1964), but also includes the various terminal regions associated with the dopaminergic projecting neurons (Andén et al., 1966; Björklund and Dunnett, 2007; Ciliax et al., 1995). More importantly for current considerations however, DAT is found heterogeneously expressed across dopamine brain

regions and even within dopamine neurons themselves (Blanchard et al., 1994; Nirenberg et al., 1997a; Salvatore and Pruett, 2012). There are meaningful differences in DAT levels found between motor and limbic compartments of the rodent forebrain (Nirenberg et al., 1997a), but moreover, the ratio of DAT to a dopamine synthesis enzyme (TH) also differs across regions (Salvatore and Pruett, 2012). These differences in part explain the significant difference in dopamine kinetics observed between various forebrain regions (Garris and Wightman, 1994; Jones et al., 1995a,b). Within a given dopaminergic neuron however, the DAT is found expressed both within the terminal area and along the axon but also around the soma and dendrites (the so called somatodendritic area; Hersch et al., 1997; Nirenberg et al., 1996b). Ultrastructurally, the DAT is well noted to be localized predominantly perisynaptically, in intracellular membranes of dopaminergic cells, and was also found on the external plasma membranes of small distal dendrites (Hersch et al., 1997; Nirenberg et al., 1996b, 1997b).

Differences are observed in the density of DAT expression (in relation to TH) between the somatodendritic areas and the axonal terminal regions (Salvatore and Pruett, 2012). Moreover, there is evidence of differences in the functional regulation (as measured via phosphorylation) of both TH and DAT between the somatodendritic area of substantia nigra and ventral tegmental area neurons and their respective terminal fields (Salvatore and Pruett, 2012). These findings support the concept of functional compartmentalization of dopamine activity between cell body areas and their axonal terminals (Ford et al., 2010).

It should be reemphasized that since the DAT is dynamically regulated via a multitude of cellular factors, its expression across brain regions or within a given cell is not static and is instead highly plastic (Mortensen and Amara, 2003; Vaughan and Foster, 2013). Examples of this plasticity can be observed from either pharmacological or environmental manipulation. Region-specific expression levels of DAT mRNA are altered after exposure to psychostimulants such as amphetamine (Kahlig and Galli, 2003; Lu and Wolf, 1997), and DAT protein levels are altered as a response to an enrichment of the environment in early development (Zhu et al., 2005). The function of DAT is to control the signaling of dopamine in the extracellular space, but in doing this the DAT directly contributes to the regulation of dopamine in the cytoplasmic compartment. Through its reuptake function the DAT provides recycled dopamine molecules which may be packaged for release even under conditions where *de novo* synthesis is inhibited and down-regulated. The contributions of DAT molecules to dopamine function, however, are dependent on the amount of neurotransmitter released and are therefore contingent on vesicular packaging (Lohr et al., 2017).

3. The vesicular monoamine transporter-2 protein: VMAT2

The protein responsible for sequestering cytosolic dopamine and packaging it into secretory vesicles is the vesicular monoamine transporter (VMAT; Eiden and Weihe, 2011; Erickson et al., 1992; Liu et al., 1992). The VMAT has two isoforms denoted as VMAT1 and VMAT2 and these are differentially expressed. Monoaminergic neurons throughout the CNS of the rat express the VMAT2 isoform (Schuldiner et al., 1995; Weihe et al., 1994). VMAT1 is only expressed in endocrine/paracrine cells associated with the stomach, intestine, and sympathetic nervous system (Peter et al., 1995; Weihe et al., 1994). Thus, only the VMAT2 isoform is of consideration in the current focus. Regardless, both isoforms belong to the SLC18 family of transporter proteins, wherein the vesicular transporter for acetylcholine is also a member (Eiden et al., 2004; Lawal and Krantz, 2013). Within dopamine neurons, VMAT2 is localized on both types of secretory vesicles (small synaptic vesicles and large dense core vesicles; Kelly, 1993; Nirenberg, Chan, Liu, Edwards and Pickel, 1997c). However, there appears to be preference between these vesicle types as their expression is not equal, likely resulting from differences in trafficking regulation (Fei et al., 2008; Nirenberg et al., 1995). Differential expression of VMAT2 on vesicle type is seen in cell type-specific manner (e.g., neurons versus neuroendocrine cells.)

and is just one example that highlights the difficulty in integrating conclusions from varying research efforts. Given the complexity of the system, an intrinsic limitation on generalizing results from *in vitro* studies (looking at VMAT2 in PC12 cells) is that they may fail to account for some of the important network factors that the functioning VMAT2 is embedded within in (Edwards, 2007; Eiden and Weihe, 2011; Kelly, 1993).

3.1. Pharmacological and genetic manipulation of VMAT2

As the name would suggest, VMAT2 is a transporter protein expressed on vesicular membranes for the monoamines (dopamine, serotonin, norepinephrine, and histamine). As such, pharmacological agents that affect its function (e.g., the antagonist reserpine) non-selectively disrupt function of all the monoamines and not just dopamine (Erickson et al., 1992; Chaudhry et al., 2008). The expression across multiple systems provides a considerable challenge to attempts to utilize the VMAT proteins as therapeutic drug targets, as the effect on the various monoaminergic systems are often difficult to segregate (for reviews see, Bernstein et al., 2014; Howell and Negus, 2014). Nevertheless, disruption of general VMAT function with reserpine produces profound parkinsonian-like behavioural effects in rats. These effects can be reversed by application of L-DOPA, a dopamine prodrug (Colpaert, 1987). This result indicates the importance of VMAT2 for dopaminergic function. Indeed, antagonism of VMAT function with reserpine and tetrabenazine (selective antagonist for VMAT2) dramatically reduced dopamine quantal release from dopamine neurons (Floor et al., 1995; Pothos et al., 1998). VMAT2 has also been found to be critical for the expression of amphetamine-induced behaviours in rodents, as demonstrated by Freyberg et al (2016). Using a novel high-affinity VMAT2 inhibitor (a derivative of dihydrotetrabenazine, (+)-CY477), they demonstrated acute inhibition of both methamphetamine and amphetamine self-administration in rats. Moreover, administration of lobelane or its analog GZ-793A (both relatively selective inhibitors of VMAT2) have been found to significantly decrease methamphetamine self-administration in rats via reduced VMAT2 function (Beckmann et al., 2012; Neugebauer et al., 2007; Nickell et al., 2017; Wilmouth et al., 2013). GZ-793A inhibition of VMAT2 was found to reduce dopamine release specifically in limbic terminal fields (e.g., nucleus accumbens shell; Meyer et al., 2013). Drugs specifically targeting VMAT proteins have been typically limited to a relatively small number of selective agents (e.g., lobelane and tetrabenazine) and their derivatives. However, the recent development of high throughput screening methods has introduced a variety of possibilities in developing novel VMAT targeting drugs (Bernstein et al., 2014). An example of one such method includes optical imaging using fluorescent false neurotransmitter as a visualizable substrate in cell cultures to examine the function of VMAT2 (Bernstein et al., 2012; Hu et al., 2013). The use of this method to design reversible and selective VMAT2 inhibitors has demonstrated viability in predicting pharmacological effect *in vivo* in rodent models (Freyberg et al., 2016). However, there are risks associated with disrupting VMAT2 function as these proteins are critical to regulation of possible neurotoxic effects associated with dopaminergic activity (Staal and Sonsalla, 2000; Vergo et al., 2007). In part due to this, VMAT proteins have featured prominently in models of neurodegenerative disorders associated with dopamine function (Liu and Edwards, 1997; Lotharius and Brundin, 2002; Pifl et al., 2014; Wimalasena, 2011).

The critical importance of VMAT2 activity for general dopaminergic function is further emphasized by genetic manipulation studies. Full VMAT knockout mice do not survive past a few days after birth (Wang et al., 1997). VMAT2-deficient mice survive, but they show profound pathology resulting from depletion of monoamines throughout the CNS (Eiden and Weihe, 2011; Lohr et al., 2017; Taylor et al., 2011). In addition, VMAT2 is evidenced to be considerably neuroprotective to dopamine neurons in a variety of toxicity models (Goldstein, 2013; Guillot and Miller, 2009; Lotharius and Brundin, 2002; Mosharov et al., 2009;

Staal and Sonsalla, 2000). This neuroprotective effect has been demonstrated using overexpression of VMAT2 in cultured cells, which attenuates aminochrome (a toxic dopamine oxidation metabolite) or methamphetamine-associated toxicity (Muñoz et al., 2012; Vergo et al., 2007). Moreover, in mice, neuroprotection against striatal methamphetamine toxicity observed following the administration of the brain-gut peptide PACAP38 appears to be associated with an increase in VMAT2 expression (Guillot et al., 2008).

This neuroprotective function of VMAT2 expression likely results from the active sequestration and removal of dopamine molecules from the cytoplasm that can be neurotoxic when oxidized (Goldstein et al., 2013; Guillot and Miller, 2009). The importance of VMAT2 in dopamine signaling is thus both enabling exocytosis but also maintaining cellular health. For these reasons in part, it should not be surprising that this transporter protein is dynamically involved in reciprocal regulations associated with quantal size of the vesicular transmitter (Edwards, 2007; Eiden and Weihe, 2011; Lohr et al., 2017).

3.2. Molecular structure and physiology of rodent VMAT2

Structurally the VMAT2 protein of the rat was found to be composed of 515 amino acids characterized by 12 transmembrane domains (Eiden et al., 2004; Erickson et al., 1992; Lawal and Krantz, 2013; Schuldiner et al., 1995). Both the N- and the C-terminals are located within the cytosol, and there are a variety of reactive residue sites (for glycosylation, phosphorylation etc.) located on these, as well as on intraluminal loops (Schuldiner et al., 1995). VMAT2 is a proton-dependent antiporter. As such, the conformational changes cause the protein to exchange a neurotransmitter from the cytoplasm for two luminal protons (Lawal and Krantz, 2013). This contrasts with the symport fashion of transport previously described for the DAT (Chen and Reith, 2000; Kristensen et al., 2011). The exchange thus relies on an electrochemical gradient that creates a driving force on protons to escape the vesicles. This gradient is established by a vacuolar H⁺-ATPase (V-ATPase) that shuttles protons into the vesicle against its concentration gradient by catalyzing ATP for energy (see Fig. 1; Inoue et al., 2005; Moriyama and Futai, 1990; Stadler and Tsukita, 1984). With a proton gradient established across the vesicular membrane, the internal lumen of the vesicle is more acidic than the cytoplasmic side (pH of ~5.5 compared to cytosol pH of ~7) and a transmembrane electrical potential (~50 mV) is established (Feldman et al., 1997; Knoth et al., 1981; Schuldiner et al., 1995). This electrogenic process thus requires the movement of anions (i.e., chloride ions) to counter the proton flux, as without this, the electrical charge opposition will effectively limit proton movement across the membrane (Moriyama and Nelson, 1987). There are chloride channels present in the vesicular membrane which allow for this anionic counter flux directly, and this process is dependent on the cytoplasmic chloride concentrations (Moriyama and Nelson, 1987). This also indicates that the electrical and pH components of the gradient are separable and may be regulated independently (Edwards, 2007; Takamori, 2016). Indeed, dopamine loading into vesicles is maximal under conditions of high cytosolic chloride concentration (Hell et al., 1990).

There are many factors associated with neuronal physiology that alter the function of VMAT2 and thereby the quantal size of vesicular transmitter (Edwards, 2007; Pereira and Sulzer, 2012; Pothos et al., 2002; Takamori, 2016). In contrast to the plasma membrane transporter, the ionic gradients utilized by the VMAT are not necessarily stable. They dissipate with the routine process of vesicular recycling and must continually be regenerated. Thus, the V-ATPase represents an additional target for vesicle regulation, though there remains a paucity of *in vivo* information in this regard (Edwards, 2007; Inoue et al., 2005; Takamori, 2016). Nevertheless, disruptions to the function of the V-ATPase or the gradient it establishes, alters the ability of VMAT2 to sequester dopamine molecules into the synaptic vesicles (Edwards, 2007; Inoue et al., 2005; Johnson et al., 1981). The kinetic rate of

VMAT2 translocation of dopamine molecules into vesicles is dependent not only on the magnitude of the pH gradient (the acidification of the intravesicular lumen) but also on the cytosolic concentration of neurotransmitter (Lawal and Krantz, 2013; Pereira and Sulzer, 2012). Some of the effects of cytoplasmic dopamine can be observed after treatment with L-DOPA, which is metabolized into dopamine within the neuron, and thereby increases levels of dopamine. L-DOPA treatment causes an increase in the size of vesicles, with vesicular numbers remaining constant, thus the absolute quantal size of the transmitter is increased (Gong et al., 2003; Pothos et al., 2002). This increase in vesicular size represents a greater potential pool of releasable neurotransmitter and emphasizes that vesicular content can be dynamically regulated.

3.3. Regulation of VMAT2 function

VMAT2 proteins may be regulated, similarly to DAT, by both expression level changes and post-translational modifications (Eiden et al., 2004; Eiden and Weihe, 2011; Schuldiner et al., 1995). Expression of VMAT2 may be altered upon exposure to drugs that modulate dopamine release dynamics (Fleckenstein et al., 2009). Such drugs may include amphetamine (Lu and Wolf, 1997), cocaine or methylphenidate (Brown et al., 2001; Sandoval et al., 2002), and apomorphine (Truong, Hanson and Fleckenstein, 2004a). These dopaminergic drugs affect different components of the dopaminergic synapse and have distinct effects on VMAT2 function and expression. As mentioned previously (see section ‘Pharmacological manipulation of the DAT’), amphetamine causes an emptying of the vesicular pool of dopamine into the cytoplasm (Sulzer et al., 1995). Using optical approaches in *Drosophila melanogaster*, VMAT2 was demonstrated to be critical in both the deacidification of secretory vesicles which accompanies amphetamine administration, as well as the subsequent increase in cytosolic concentration (Freyberg et al., 2016; Hiranita and Freyberg, 2016). The presence of the VMAT2 protein allows for amphetamine to be transported across the secretory vesicle membrane, and this movement is coupled with proton-antiport. Thus, as amphetamine travels as a substrate, there is an ensuing alkalization of the intra-vesicular space actively performed by the VMAT2 proteins (and thus a disruption of the concentrative force derived from the pH-gradient). This effect on vesicular storage is characteristic of the DAT-reversing drugs and is not found with DAT-blocking drugs such as methylphenidate (Freyberg et al., 2016).

Additionally, early developmental experience was found to alter VMAT2 expression (Barr et al., 2009). Increases in VMAT2 expression on vesicular membranes, as would be expected in a steady-state model, increases the quantal size and the resulting amount of dopamine released (Lohr et al., 2014; Pothos et al., 2000). But interestingly, this increased VMAT2 expression also increases the frequency of release events (Pothos et al., 2000). There is evidence that vesicular transporter expression can be upregulated or downregulated in an activity-dependent fashion. This in turn alters the quantal size and synaptic efficacy for a given neurotransmitter (De Gois et al., 2005; Eiden and Weihe, 2011). Although as previously mentioned, there are pharmacological methods of inhibiting VMAT2 function, there are few methods of positively manipulating VMAT2 function (Lohr et al., 2017; Oshervich, 2014). One of the only tools currently available for *in vivo* investigations has been to genetically over-express VMAT2 (Lohr et al., 2014, 2015, 2017). This elevated expression translates to increased vesicular size and extracellular dopamine.

The expression of VMAT2, as with DAT, is a representative product of the complex interplay between transcriptional factors, protein kinases, heterotrimeric G-proteins, and binding-partner interactions (Eiden and Weihe, 2011; Fei et al., 2008; Watson et al., 2001; Yao et al., 2004). An example of this interplay may be seen with the VMAT2 dependence on protein kinase A (PKA) signaling for appropriate membrane trafficking (Yao et al., 2004). Though critically involved, PKA does not appear to directly phosphorylate the VMAT2 protein but

instead regulates N terminal glycosylation and directly phosphorylates cAMP response element-binding protein (CREB) for trafficking and transcriptional activation, respectively (Watson et al., 2001; Yao et al., 2004). In addition to expression, the rate of VMAT2 function may be altered by regulatory interactions. For instance, there appears to be a role of heterotrimeric G-proteins in inhibiting VMAT2 function (Edwards, 2007; Höltje et al., 2000). This association is dependent on an intraluminal loop, though the exact mechanism of regulation is still unclear (Brunk et al., 2006).

In summary, VMAT2 is a vesicular membrane-localized protein responsible for packaging dopamine, among the other monoamines, into secretory vesicles (Lawal and Krantz, 2013). VMAT2 is a critical component in the compartmentalization of dopamine and is directly related to determining quantal size (Eiden and Weihe, 2011; Pereira and Sulzer, 2012). Its function is dependent on a transmembrane electrochemical gradient established by a V-ATPase, with a primary importance of the pH component for dopamine shuttling (Hell et al., 1990; Schuldiner et al., 1995). The function and expression of the VMAT2 protein is dynamically regulated and subject to modulation, which thus directly affects vesicular stores of dopamine (Pothos et al., 1998, 2000, 2002).

4. DAT and VMAT2 proteins as regulators of dopamine neurotransmission

Within dopaminergic neurons, both the DAT and the VMAT2 proteins function in concert to appropriately compartmentalize dopamine in relation to its signaling activity (German et al., 2015; Lohr et al., 2017; Uhl, 1998). These proteins possess distinct and separable roles in this regard however, but the interplay and interaction between them is a key regulator of dopamine quantal size (Lohr et al., 2017; Pereira and Sulzer, 2012; Schmitz et al., 2003).

Most interest in DAT-to-VMAT ratios has been for models of neuroprotection involved in the study of parkinsonian neurodegeneration (Guillot and Miller, 2009; Lohr et al., 2014, 2017; Uhl, 1998). This is because of the critical role that both proteins play in regulating the potentially neurotoxic levels of cytosolic dopamine (Masoud et al., 2015; Miller et al., 1997; Mosharov et al., 2009; Pifl et al., 2014). Elevated levels of DAT relative to VMAT2 proteins translate to lower extracellular levels and presumably increased cytosolic levels of dopamine (see Fig. 1). This accumulation of dopamine produces oxidative stress (via monoamine oxidase production of reactive metabolites or even dopamine auto-oxidization), that damages the neuron, possibly even causing cell death (Masoud et al., 2015; Mosharov et al., 2009). Elevated levels of VMAT2 relative to DAT are therefore neuroprotective because they sequester the dopamine molecules and prevent their oxidation and the production of free radical species (Guillot and Miller, 2009). However, elevated levels of VMAT2 also translate to higher extracellular dopamine levels (Lohr et al., 2014), thus there are constraints imposed on the regulation of the synaptic signaling properties (the DAT proteins present will be saturated and unable to recycle and limit the diffusion of dopamine molecules). The DAT and VMAT2 roles can thus be conceptualized as oppositional in determining cytoplasmic dopamine levels. The interaction between them is critical in determining the net output of the dopaminergic synapse (levels of released dopamine able to act extracellularly on heteroreceptors). Changes in dopamine levels may in turn profoundly alter postsynaptic receptor-dependent molecular events. There is evidence that even subunit assemblies, which are necessary for receptor-dependent signaling cascades, may be altered in relation to levels of dopamine binding (such as the olfactory type G-protein α subunit; Hervé, 2011; Ruiz-DeDiego et al., 2015). These changes in functional subunit expression may provide negative feedback, whereby, an increased amount of receptor activation due to increased dopamine levels and may consequently reduce the rate and extent of postsynaptic signaling cascades.

One central aspect for consideration of this net output is the quantal

size of secretory vesicles and how it may be dynamically regulated by VMAT2 and DAT proteins.

4.1. DAT, VMAT2, and dopamine quantal size

Inherent in the term quantal size is both the amount of transmitter stored in vesicles and the amount of transmitter released during vesicular fusion with the plasma membrane, two processes which may be regulated separately (Pereira and Sulzer, 2012). The question of the role of both DAT and VMAT2 proteins in relation to quantal size considered in this paper is how these proteins contribute to the amount of transmitter stored within the secretory vesicles assuming a steady-state model. This idea of a steady-state model for vesicular loading assumes that an increase of transmitter packaging (above and beyond any outflow rate) accomplished by VMAT2 function would mediate an increase in quantal size (Williams, 1997).

As previously described, the DAT is the primary element for regulating the spatial and temporal extent of dopamine signaling in the extracellular space (Jones et al., 1998, 1999). Based on modeling data it has been argued that, alternatively to simply terminating dopamine signaling, the DAT more actively functions to shape dopamine signaling dynamics in relation to amount of transmitter released (Cragg and Rice, 2004; Rice and Cragg, 2008). As such, alterations to the amount of dopamine released (e.g. changes in quantal size) directly influence its modulatory effect on extrasynaptic diffusion of transmitter molecules. The amount of transmitter packaged into vesicles was initially assumed to be a static and uninfluenced feature of neurotransmission within the nervous system (Boyd and Martin, 1956; Liley, 1956). However, it is now widely recognized that transmitter packaging is governed in a dynamic fashion and may be increased or decreased (for reviews see Pereira and Sulzer, 2012; Reimer et al., 1998; Takamori, 2016).

The high levels of transmitter concentration found within the intraluminal vesicular space represent a barrier that the function of the VMAT2 proteins must overcome (Blakely and Edwards, 2012). As described for VMAT2 proteins their loading of transmitter into vesicles is primarily driven by the proton gradient established and maintained across the vesicular membrane by the function of V-ATPase. Increases to the magnitude of this proton gradient across the vesicular membrane (i.e. acidification of the intravesicular space) increases the driving force of transmitter loading and thereby the quantal size (Pereira and Sulzer, 2012; Takamori, 2016). This so called 'hyperacidification'-driven increase in quantal size has been demonstrated for catecholamines in culture chromaffin cells (Markov et al., 2008; Pothos et al., 2002), but also *in vivo* in both *Drosophila* and mice (Aguilar et al., 2017). This observed increase in acidification occurs in response to direct stimulation and depolarization of the cell, however, the exact mechanisms remain an active avenue of research (Aguilar et al., 2017; Rossano et al., 2017). In contrast, disruption to this proton gradient, as observed after administration of *D*-amphetamine or methamphetamine, greatly depletes observed intravesicular transmitter content (Freyberg et al., 2016). Importantly for consideration of the role of VMAT2, it has been demonstrated for large dense core vesicles in chromaffin cells that as transmitter content within the vesicle increases so does its volume and as a result the concentration gradient acting on the transmitter remains constant (Colliver et al., 2000; Gong et al., 2003). Thus, the function of VMAT2 proteins in loading transmitter into secretory vesicles does not appear to be limited by an ever-increasing concentration gradient. This suggests that once sufficient acidification has been established by V-ATPase the function of VMAT2 proteins may be considered a critical mediator of increasing the quantal size of dopamine (Blakely and Edwards, 2012; Colliver et al., 2000).

An increase in quantal size mediated by VMAT2 function constitutes an increase in released transmitter, which may serve to extend the spatiotemporal volume of dopamine signaling in the extracellular space (Schmitz et al., 2003; Periera and Sulzer, 2012). Since this functional aspect of dopamine signaling is primarily regulated by the activity and

expression of DAT molecules, the effect of quantal size on dopamine neurotransmission interacts directly with the DAT (Cragg and Rice, 2004; Sotnikova et al., 2006). It should be noted that physical associations between DAT molecules and secretory vesicles are an additional form of functional relation between DAT proteins and quantal size (Egaña et al., 2009). Such physical associations allow for DAT molecules to increase the vesicular contents in the recycling pool of vesicles.

4.2. Pharmacological delineation of DAT and VMAT2 function

Pharmacological interventions that modulate various components of the signaling dynamics of dopamine provide the basis for some speculation on the interplay between DAT and VMAT2 (Eiden and Weihe, 2011; Vaughan and Foster, 2013; Zhu and Reith, 2008). As mentioned previously, drugs such as cocaine, amphetamine, and apomorphine all affect the expression and function of these transporter proteins. These drugs possess distinct pharmacodynamics that potentially reveal information about the interactions between DAT and VMAT2 in their roles of regulating dopamine compartmentalization. Generally, dopamine releasing drugs (e.g., the amphetamines), and dopamine reuptake inhibitors (e.g., cocaine), differentially affect VMAT2 function (Fleckenstein et al., 2009; German et al., 2015).

The dopamine releasing drugs produce a decrease in VMAT2 function that lasts past their pharmacological action while the reuptake inhibitors produce the opposite effect and increase VMAT2 function (Eiden and Weihe, 2011; Fleckenstein et al., 2009). The potent dopamine releasing drug methamphetamine has repeatedly been found to produce a reduction in general VMAT2 function (Guilarte et al., 2003; Hogan et al., 2000). Similar findings with *D*-amphetamine administration support the idea that dopamine releasing drugs produce a lasting effect of decreased VMAT2 function (Schwartz et al., 2007). This decrease is likely a result of a redistribution of VMAT2 proteins in response to changes in cytosolic dopamine concentration as it is not observed following cocaine or methylphenidate administration (Riddle et al., 2007; Schwartz et al., 2007). However, it is not always the case that DAT-reversing drugs such as methamphetamine inhibit VMAT2 function. Markov et al. (2008) found evidence in cultured cells that longer-term exposure to methamphetamine actually appears to cause an increase in acidification of intraluminal vesicular space and an associated increase in quantal size.

The DAT inhibitors (cocaine and methylphenidate) prevent reuptake of extracellular dopamine but leave VMAT2 function intact thereby reducing cytoplasmic levels of dopamine (Fleckenstein et al., 2009). In contrast to the releasers, the administration of reuptake inhibitors appears to increase VMAT2 function and expression (Brown et al., 2001; Schwartz et al., 2007).

The effect of high cytoplasmic dopamine levels, as previously stated, has neurotoxic effects on the cell. Inhibition of VMAT2 function with reserpine prior to methamphetamine administration amplifies the oxidative stress observed (Thomas et al., 2008). Thus, the differential inhibition on the transporters critically affects the cytoplasmic compartment of dopamine in two opposite directions. These results indicate that VMAT2 function may be directly regulated by cytoplasmic levels of dopamine (Eiden and Weihe, 2011; Fleckenstein et al., 2009).

4.3. Function protein associations involving DAT and VMAT2

It must also be noted that there appears to be involvement of dopamine receptors in mediating the effects of both releasers and reuptake inhibitors on the observed changes to VMAT2 function (Fleckenstein et al., 2009). Antagonism of D_2 receptors mitigated the effects of both dopamine releasers and reuptake blockers (Brown et al., 2001, 2002). Conversely, activation of D_2 receptors increased VMAT2-mediated dopamine uptake (Truong, Newman, Hanson and Fleckenstein, 2004b; Truong et al., 2004a). As previously mentioned,

DAT proteins have direct functional interactions with D₂ receptors (Lee et al., 2007), but there is also evidence that DAT proteins can form functional complexes with secretory vesicles (Egaña et al., 2009). Indeed, VMAT2 proteins themselves may additionally form functional associations with TH and AADC enzymes (Cartier et al., 2010). Thus, secretory vesicles and the molecular regulation of their packaging represents an important direct point of interaction between synthesis and transmitter clearance. As noted previously, the direct complexing of synthesis-associated enzymes and secretory vesicles may mean that synthesis largely avoids contributing to cytoplasmic pool size of dopamine (Chen et al., 2003). This coupling of synthesis and packaging occurs through the function of VMAT2 proteins (Cartier et al., 2010). To the degree that this occurs *in vivo*, it would presumably lessen the contribution of the synthesis pathway in determining cytosolic concentration of dopamine molecules. As a result, it is likely that the process of DAT-mediated dopamine reuptake would then comprise the main contribution to cytoplasmic transmitter pools when the DAT proteins are not coupled with secretory vesicles.

These findings of protein interactions indicate that modulations to DAT function can rapidly and reversibly alter VMAT2 function, which in turn alters quantal release of dopamine (Pothos et al., 2000). A lower level of DAT function coinciding with a normal functioning of VMAT2 increases the levels of extracellular dopamine (Lohr et al., 2014, 2017). The intricate oppositional roles that DAT and VMAT2 have in relation to compartmentalizing dopamine and preventing possibly catastrophic neurotoxicity raise the interesting question of why their relative expression levels may differ in a systematic fashion.

4.4. Expression differences between DAT and VMAT2 in the rodent brain

Among the molecular and functional heterogeneity of the midbrain dopamine complex of the rodent brain, there is evidence of systematic regional differences in the expression of DAT and VMAT2 mRNA (Brown et al., 2013; Lammel et al., 2008; Li et al., 2013). Generally, these mRNA differences are found along a medial-to-lateral gradient, within the regions of the ventral mesencephalon, with differences resulting largely from alterations in DAT expression (Lammel et al., 2008; Li et al., 2013). However, Brown and colleagues (2013) failed to replicate the same pattern of results using laser-microdissected individual cells. These latter researchers did not observe differences in DAT expression relative to VMAT2 but did find regional differences in DAT expression relative to TH. However, the authors suggest this may result from interspecies variation (Brown et al., used mice while Li et al., and Lammel et al., both used rats). The gradient differences in DAT expression (relative to both TH and VMAT2) likely reflect different functional circuits embedded in the complicated input-output architecture of the midbrain dopamine complex (Beier et al., 2015; Lammel et al., 2008, 2012; Lammel et al., 2011; Lammel et al., 2014). These findings are consistent with earlier work that identified heterogeneity in both mRNA and protein expression of DAT and VMAT2 across midbrain regions in both rodents and humans (Blanchard et al., 1994; Hurd et al., 1994; Pickel et al., 1996). Nirenberg et al. (1996a,b, 1997a,b) investigated VMAT2 protein expression in the substantia nigra and ventral tegmental regions of the rodent midbrain. Using immunogold labeling, higher levels of VMAT2 protein per unit of area were found in dendrites of the VTA in comparison with the substantia nigra pars compacta or reticulata (Nirenberg et al., 1996a). For DAT protein immunoreactivity in the midbrain, the reverse pattern has been found with a caudoventrolateral to rostradorsomedial gradient being demonstrated in the rat midbrain (Freed et al., 1995; González-Hernández, Barroso-Chinea, de la Cruz Muros, del Mar Pérez-Delgado and Rodríguez, 2004). Thus, generally, higher DAT levels appear in the substantia nigra in comparison with the VTA. These findings of heterogeneity within the midbrain for VMAT2 and DAT expression likely reflect different somatodendritic release profiles of neurons within these regions (Nirenberg et al., 1996a,b, 1997b; Pickel et al., 1996).

At the level of the striatum (an exemplary dopamine terminal area), there is considerable regional heterogeneity in DAT and VMAT2 expression (Marshall, O'dell, Navarrete and Rosenstein, 1990; Masuo et al., 1990; Nirenberg et al., 1997a). Radiolabeled binding studies indicated that the pattern of striatal expression of VMAT2 and DAT proteins generally parallel each other, with highest expression found where dopamine innervation is greatest (Marshall et al., 1990; Masuo et al., 1990). However, there is evidence that the ratio of the two proteins exhibits some regional heterogeneity within the striatum. Radiolabeled dihydrotetrabenazine and GBR 12783, to identify VMAT2 and DAT expression, respectively, found that their relative proportions differed within the striatum along a rostrocaudal gradient (Leroux-Nicollet and Costentin, 1994). The rostral portion of the striatum (corresponding to projections from the ventromedial midbrain dopamine complex) indicated a higher proportion of VMAT2 protein relative to DAT (Leroux-Nicollet and Costentin, 1994). Moreover, immunohistochemistry and western blotting data support the idea that striatal VMAT2 expression relative to DAT is highest along a ventromedial-to-dorsolateral gradient (González-Hernández et al., 2004). Less DAT protein appears to be expressed in the ventromedial portions of the rodent striatum in comparison with the dorsolateral regions. This heterogeneity may even extend to subregional divisions within the striatum as DAT protein appears to be expressed at lower levels in the nucleus accumbens shell compared with core in the rat (Nirenberg et al., 1997a).

Indeed, it is known that the striosome and matrix compartments within the striatum have functional differences in dopamine release (for review see, Brimblecomb and Cragg, 2016). This relationship of release to compartment does appear to vary across the ventral and dorsal portions of the striatum with higher release associated with the matrix compartment in dorsal striatum but this relationship is reversed in the ventral portion (Brimblecomb and Cragg, 2015, 2016; Salinas et al., 2016). However, the extent of contribution and the specific role of the dopamine transporters in relation to this difference in dopamine release is yet to be resolved. Evidence of differential expression across compartments indicates a possible role for the DAT, however, there are inconsistencies depending on methods used as to the nature of this association. Immunoreactivity finds higher DAT levels within striosomes compared to matrix (Freed et al., 1995; Salinas et al., 2016). While autoradiography suggests the opposite, with DAT levels greatest in matrix compared to striosome (Graybiel and Mortalla, 1989). Additional complexity is revealed by Salinas et al. (2016) findings which indicated that while different in immunoreactivity levels, the functional kinetics associated with dopamine uptake were similar across striosome and matrix compartments. However, this finding did not extend to the ventral striatum, where the striosomes have evidently greater dopamine release compared with matrix (Salinas et al., 2016). The evidence of VMAT2 localization to striatal compartments is less clear, though there is some indication it may be enriched in human matrix, offering some neuroprotection in parkinson's disease (Miller et al., 1999). The full role of both VMAT2 and DAT in mediating these striosomal and matrix compartment dynamics appears unknown. However, a systematic investigation and consideration of the proportions of VMAT2 and DAT inherent within these striatal compartments, represents much potential for elucidation.

It is suggested that these molecular expression differences between subpopulations of neurons reflect meaningful functional characteristics. The exact nature of how these molecular characteristics map onto projection-specific functions from the midbrain dopamine complex remains an exciting avenue for future research.

One possible functional consequence of these molecular characteristics is that they allow for particular subcomponents of the ascending mesolimbic projections to differ in their capacity for signaling in both magnitude and duration. The subcomponent of the ascending midbrain dopamine projections associated with positive emotional arousal (for greater detail see Brudzynski et al., 2018), may be such a candidate.

The possession of a greater number of VMAT2 protein expression relative to DAT expression in the mesolimbic terminals arriving to the forebrain may enable these projections to sustain a greater duration of signaling. This occasional increase in dopaminergic signaling would thereby be producing a sustained emotional arousal and more powerful emotional state in highly emotional situations.

5. Conclusion

In conclusion, the DAT and VMAT2 proteins both contribute to the overall signaling properties of dopamine at the synaptic terminal. These proteins, and the possible functional ratios between them, represent useful molecular markers for studies across a range of important neuroscience fields. The expression of DAT and VMAT2 has been recently utilized in investigations of human psychiatric disorders (e.g., schizophrenia; Purves-Tyson et al., 2017). Moreover, these proteins undergo alteration in response to drugs recruiting the mesolimbic dopamine system (e.g. morphine; García-Pérez et al., 2016) and thereby center prominently in models of addiction (German et al., 2015) and chronic treatment. These proteins are also targets for novel pharmacological interventions being studied to address psychostimulant abuse (Nickell et al., 2014). However, investigating the functional ratios of these proteins and their mutual regulatory function over dopamine signaling remains an expansive area of great interest.

Conflicts of interest

Declarations of interest conflicts: none.

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