The vesicular monoamine transporter 2: an underexplored pharmacological target

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Abstract
Active transport of neurotransmitters into synaptic vesicles is required for their subsequent exocytotic release. In the monoamine system, this process is carried out by the vesicular monoamine transporters (VMAT1 and VMAT2). These proteins are responsible for vesicular packaging of dopamine, norepinephrine, serotonin, and histamine. These proteins are essential for proper neuronal function; however, compared to their plasma membrane counterparts, there are few drugs available that target these vesicular proteins. This is partly due to the added complexity of crossing the plasma membrane, but also to the technical difficulty of assaying for vesicular uptake in high throughput. Until recently, reagents to enable high throughput screening for function of these vesicular neurotransmitter transporters have not been available. Fortunately, novel compounds and methods are now making such screening possible; thus, a renewed focus on these transporters as potential targets is timely and necessary.

Vesicular monoamine transporters: overview
The vesicular monoamine transporters (VMATs) are part of the Major Facilitator Superfamily (MFS) and the solute carrier family of transporters (SLC) subfamily. Like other MFS family members, VMATs contain 12 transmembrane spanning domains, with cytosolic C- and N- terminals and large glycosylated intravesicular loops. Members of the SLC18 subfamily are Drug:H+ antiporters; these transporters exchange intravesicular protons for extravesicular neurotransmitter.

The vesicular monoamine transporters are essential for proper monoaminergic neurotransmission, which requires the sequestration of transmitter into synaptic vesicles by VMAT for subsequent Ca2+-stimulated exocytotic release (1). This critical function is

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accomplished by the secondary active transport of neurotransmitters against their concentration gradient into synaptic vesicles (2). As proton exchangers, VMATs rely on the proton gradient generated by the V-type ATPase across the vesicular membrane and the import of chloride via the CIC-3 chloride channels. The high concentration of intravesicular protons allow for the exchange of two protons for each molecule of neurotransmitter transported (3, 4). VMATs primarily transport monoamines (dopamine, serotonin, norepinephrine and histamine), but also sequester toxicants into vesicles, shunting them away from cytosolic sites of action (5–12). This is particularly interesting given sequence homology between VMATs and the bacterial toxin extruding antiporters (TEXANs) (13).

In mammals, there are two VMAT isoforms. VMAT1 (SLC18A1) is expressed exclusively in the periphery, with expression in the sympathetic nervous system, adrenal chromaffin cells, and endocrine/paracrine cells of the gut. VMAT2 (SLC18A2) has both peripheral (enteric nervous system, adrenal chromaffin cells, and endocrine cells of the stomach, and platelets) and central nervous system (all monoaminergic neurons of the brain) expression (14). The transporters share common substrates with the exception of histamine, which is believed to be preferentially packaged by VMAT2.

Vesicular monoamine transporters in disease

Many neurological and psychiatric disorders can be linked to dysfunction of monoaminergic systems, including Parkinson’s disease (PD), Huntington’s disease, ADHD, dystonia, schizophrenia, addiction, and depression (15–20). Although the origin of monoaminergic dysfunction varies, manipulation of vesicular function could be a useful target for modulating monoamine homeostasis. Data from our lab and others suggests that direct modification of monoamine vesicular function may be beneficial in a variety of disorders, either in isolation or in conjunction with existing therapies. For purposes of this review, we will focus on dopamine packaging by VMAT2 and PD, which has been the focus of work in our lab. Data from many labs have demonstrated that proper packaging of dopamine into vesicles is critical since cytosolic dopamine is neurotoxic. Cytosolic dopamine is metabolized by enzymatic deamination or broken down by autooxidation, producing reactive, harmful oxidative products (21–28). Efficient transport of dopamine by VMAT2 prevents accumulation of these toxic byproducts.

Toxicological disruption of vesicular transport

As explored in our recent review, “Vesicular Integrity in Parkinson’s Disease,” many insults, both environmental and genetic, that lead to PD converge on vesicle function (21). Several classes of environmental toxicants, including pesticides, polychlorinated biphenyls, and brominated flame retardants, have been associated with PD pathology (21, 29–31). Epidemiological evidence linking these toxicants to disease risk is extensive (32–43). Additionally, mechanistic studies have demonstrated that these compounds exert selective toxicity to dopaminergic neurons via inhibition of synaptosomal and vesicular uptake of dopamine and resultant oxidative stress (44–60).
In vitro and animal models of modified vesicular transport

Many studies in both in vitro and animal models have also demonstrated that unregulated cytosolic dopamine is neurotoxic (61–64). In vitro experiments suggest that the relative vulnerability of dopamine neurons in PD may be mediated by cytosolic dopamine (65). Furthermore, mice that express DAT on non-dopaminergic striatal neurons, which lack VMAT2, take up dopamine into those neurons, but do not store it in vesicles, producing motor deficits and profound striatal neurodegeneration, accompanied by markers of increased dopamine oxidation (66). Additionally, transgenic mice with altered expression of VMAT2 have illustrated the critical nature of vesicular storage of dopamine for the integrity of the nigrostriatal system. VMAT2 knockout mice die soon after birth, while heterozygotes develop normally, but display increased sensitivity to amphetamine, and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (12, 67, 68). Mice that are hypomorphic for VMAT2 (~5% wild type expression) have been developed as a mouse model of PD (10, 69–71). These mice develop normally, but undergo progressive nigrostriatal degeneration, α-synuclein accumulation, show markers of oxidative stress, and develop motor and nonmotor symptoms of PD when they express alpha-synuclein (10, 69, 70).

In addition, genetic mutations linked to PD often affect synaptic vesicle function, leading to deficits in trafficking, transmitter storage and release. Alpha-synuclein has long been known to bind to phospholipids on the vesicle membrane (72–75). While its function is unknown, genetic ablation of the synuclein genes increases dopamine release (76, 77). In addition, dopamine influences the utilization of alternate alpha-synuclein transcripts, resulting in changes in localization of alpha-synuclein (78). Fibrillization of alpha-synuclein is promoted by oxidized dopamine; in turn, fibrilar alpha-synuclein can permeabilize the vesicular membrane, leading to further increases in cytosolic dopamine and thus more oxidative stress, creating a positive feedback loop (79). Deficiency of PINK1 function reduces synaptic efficiency by immobilizing synaptic vesicles of the reserve pool [Morais 2009]. DJ-1 interacts with synaptic vesicle proteins such as synaptophysin and Rab3A and influences the expression of VMAT2 (80, 81). Finally, DJ-1, PINK1 and parkin knock-out mouse models all show substantial presynaptic deficits in dopamine release (82–85). Together, these data suggest that vesicular dysfunction is a convergence point for both genetic and environmental risk factors of PD.

Current therapeutics targeting VMAT2

Despite the recognized importance of the vesicle in dopaminergic disease, few FDA approved drugs directly and specifically target the vesicle. Two VMAT2 inhibitors, reserpine and tetrabenazine (TBZ), have demonstrated efficacy in the treatment of disease. Although other drugs, such as amphetamine and methylphenidate, are known to affect VMAT2 function, these drugs have a complicated pharmacology due to their interaction with plasmalemmal transporters and inhibition of neurotransmitter metabolism (9, 86). As they do not exclusively act at VMAT2, we have focused on reserpine and TBZ, which specifically target the vesicle.
Reserpine

Reserpine, an alkaloid isolated from the Indian snakroot *Rauwolfia serpentine*, was introduced to Western medicine in 1952, and was widely prescribed for its antihypertensive and antipsychotic properties (87). Despite these beneficial effects of reserpine, side effects were described as resembling a parkinsonian syndrome, with symptoms including depression, gastric dysmotility, and extrapyramidal symptoms (88). Although the molecular target of reserpine was not identified for decades, researchers observed that reserpine depleted dopamine in biological tissue and caused parkinsonism in rats (89, 90). The later discovery that reserpine is an irreversible and non-specific VMAT1/2 inhibitor provided a mechanistic explanation for the effects of this compound (5, 6, 91). The anti-hypertensive effect results from VMAT2 inhibition in the sympathetic nervous system and chromaffin cells, reducing sympathetic tone and, in turn, reduced blood pressure (92, 93). Despite its effectiveness for treating hypertension, the inhibition of VMAT2 within the CNS causes the aforementioned deleterious symptoms, including severe depression (88, 94). These effects are particularly problematic given the irreversible nature of the drug, since washout requires new protein synthesis and can take several weeks (95). Due to these side effects, reserpine is no longer commonly prescribed.

Tetrabenazine

TBZ is a reversible and specific VMAT2 inhibitor that was recently FDA approved for the treatment of Huntington’s disease (HD) (96–99). HD is characterized by hyperkinetic movement caused by abnormal and increased dopamine release (100). Treatment of HD with TBZ ameliorates aberrant movement through VMAT2 inhibition and depletion of dopamine (101). TBZ also shows efficacy for the treatment of other hyperdopaminergic disease states, such as Tourette’s syndrome (101). However, as with reserpine, VMAT2 inhibition leads to depletion of other monoamines, particularly serotonin, which induces a plethora of nondopaminergic effects, including depression, parkinsonism, fatigue, and GI disturbances (99, 102, 103). While many of these adverse effects are less severe and more manageable than the side effects of reserpine, due to the reversible nature of TBZ action, the therapeutic application of TBZ remains limited (99, 102, 103).

Vesicular transporters as targets for drug development

The majority of drugs used to treat PD and other monoamine disorders target receptors or plasma membrane transporters. While current treatments for PD, such as L-DOPA and dopamine agonists, may compensate temporarily for reduced endogenous dopamine signaling, they do not promote normal neurotransmission, maintain neuronal integrity, or prevent the progression of degeneration. In addition, long-term modulation of these targets often alters receptor sensitivity resulting in loss of effectiveness and increased side effects. For example, treatment with L-DOPA or other dopamine receptor agonists induce supersensitivity of D1 receptors and may contribute to side effects experienced by PD patients after chronic treatment (104).

In contrast, direct targeting of the vesicle, alone or in combination with existing therapies, may allow for modulation of neurotransmission while maintaining the proper kinetics of
exocytotic release and termination of the signal. Such a strategy may prevent side effects and/or the loss of efficacy that result from compensatory changes in receptor sensitivity caused by the increased duration of transmitter in the synaptic cleft or increased receptor occupancy. In PD specifically, enhancing vesicular function may enhance dopamine transmission and confer resistance to further dopaminergic toxicity and cell death. Research suggests that pharmacological agents that indirectly increase VMAT2 function are neuroprotective. Pramipexole (a D2 agonist) and apomorphine (a D1/D2 agonist) enhance vesicular uptake of dopamine and may be neuroprotective (105, 106). Methylphenidate increases vesicular DA uptake in rats and prevents persistent dopaminergic deficits induced by high-dose methamphetamine administration (107, 108). Furthermore, the pituitary adenyl cyclase activating polypeptide, 38 amino acids (PACAP38) is protective against oxidative stress and dopaminergic cell damage induced by methamphetamine, likely by increasing VMAT2 expression in the striatum (109). These treatments do not directly modulate VMAT2; instead, they most likely act through upregulation of VMAT2 levels. However, the neuroprotection provided by this mechanism still suggests that pharmacological enhancement of vesicular function may both slow PD progression and improve dopaminergic function in PD patients.

Consistent with this, data from Drosophila models also suggests that enhancement of vesicular transport is a promising therapeutic target. Flies that overexpress the Drosophila isoform of VMAT in dopaminergic and serotonergic neurons show no gross defects; they are viable, grow normally, and have a typical life span (110). These flies have minor phenotypic changes including increases in stereotypic grooming behaviors and locomotion that can be reversed by reserpine, prolonged courtship behavior and decreased fertility. Furthermore, a screen for compounds that increase vesicular transport in flies with low levels of VMAT identified compounds that reversed the locomotor defect of these flies via a VMAT-dependent mechanism (111). Together, this suggests that enhancing VMAT2 function for the treatment of movement or psychiatric disorders is a strategy worth pursuing because there is no evidence, as yet, that this approach will cause major side effects.

Despite the potential utility of targeting VMAT2 to modulate vesicular function, only a few labs are developing novel ligands for this transporter. Derivatives of lobeline, ketanserin and TBZ, compounds known to bind and inhibit VMAT2, have been developed and tested for their ability to bind and/or inhibit VMAT2 (112–117). Until very recently, because of the methods and reagents available (see below), high throughput screening of vesicular transport function has not been possible. Thus, testing new compounds has been limited to small scale, derivative-based compound development for identification of more potent or specific inhibitors, rather than true high throughput screening, which enables investigation across a variety of chemical structures. Truly novel drugs cannot be identified without such screening. Novel VMAT2 inhibitors that are specific to each monoamine (i.e. a serotonergic specific VMAT2 inhibitor) may have improved efficacy for the treatment of hypermonoaminergic disorders. Additionally, direct or indirect enhancers of VMAT2 may prove highly beneficial in the treatment of hypomoanoaminergic disease, such as PD.
Current methods to measure uptake by vesicular neurotransmitter transporters

Until very recently, methods for measuring the activity of vesicular neurotransmitter transporters were not amenable to the high throughput analysis that enables drug development. Vesicular transport is typically measured by radioactive neurotransmitter uptake into vesicles isolated from rat or mouse brain tissue. Vesicles can be prepared from animals treated with various drugs or toxicants to determine the systemic effect on uptake (29, 118–120). Alternatively, to determine pharmacokinetics, isolated vesicles from untreated animals can be treated directly with the drug or toxicant of interest. While this is a powerful technique, these experiments require a large amount of tissue that necessitates the use of many animals and radioactivity. This, combined with the labor intensive and time consuming protocol for vesicle isolation and the requirement for freshly prepared vesicles, make it extremely impractical to adapt this gold standard assay for high throughput screening.

Radioligand binding assays have been used to identify compounds that competitively inhibit tetrabenazine binding (112, 114, 115). The use of radioactivity limits the adaptation to high throughput screening. Additionally, binding assays only identify compounds that directly bind VMAT2 and only if the binding inhibits tetrabenazine binding by either binding the same site or stabilizing the protein in a conformation incompatible with TBZ binding. However, it is possible that compounds that bind to sites on VMAT2 without affecting TBZ binding may modulate its function. Furthermore, modulation of other vesicular proteins may indirectly affect VMAT2 function. For example, modulation of the V-type ATPase, which establishes the proton gradient that drives VMAT2 activity, would alter vesicular function. An assay that allows for direct screening of transport function would allow for identification of compounds that alter VMAT2 function both directly and indirectly and will be a useful tool for development of novel drugs that target vesicular transporters.

Two techniques for measuring vesicular uptake in cell lines expressing the transporters of interest bypass these limitations. In the first method, cells are treated with detergent to permeabilize the plasma membrane while leaving the vesicle membrane and transport machinery intact (7). In the second method, a post-nuclear fraction is isolated from cell lines expressing the vesicular transporter of interest (121, 122). Unfortunately, these assays are also not amenable to high throughput screening. In the permeabilization method, multiple, delicate washing steps are required that would be difficult to perform successfully with a high throughput liquid handler. In the fractionation protocol, adhering the isolated vesicle fraction to a plate is technically challenging. Furthermore, the use of radioactivity itself hinders adaptation to a high throughput format. An assay that enables high throughput screening would ideally use a fluorescent reagent and not require complicated fractionations or multiple wash steps.
Novel methods to enable high throughput screening

A fluorescent assay for VMAT2 transport

Our lab and others have been working to develop high throughput screening techniques for transport function in VMAT2 containing vesicles. We recently reported the development of a fluorescent assay for measuring VMAT2 function (123). The assay utilizes the Neurotransmitter Uptake Assay from Molecular Devices, which consists of a proprietary fluorescent dye that is transported by monoamine transporters and an impermeable masking dye that blocks extracellular fluorescence. While the identity of the dye is proprietary, based on its properties and behavior in the assay, it is likely to be 4-(4-dimethylamino) phenyl-1-methylpyridinium (APP+) (124). While others have determined that APP+ does not function as a fluorescent false neurotransmitter (FFN) and cannot be used to assess release kinetics like the FFNs, it is sufficient for the purposes of measuring uptake in this artificial system (125). Similarly, we have also determined that the dye in the assay is not released from vesicles in response to potassium or amphetamine, indicating that it also does not function as an FFN (data not shown). The Molecular Devices assay is marketed for assessment of transport by the monoamine plasma membrane transporters (DAT, NET and SET) using a plate reader. To adapt this assay for measuring VMAT2 function, we created a line of human embryonic kidney (HEK) cells that stably co-express human DAT and a human VMAT2-mCherry fusion protein (123). We demonstrated that the dye localizes to VMAT2-positive compartments and that this localization is inhibited by TBZ, confirming VMAT2-mediated transport.

To quantify dye accumulation in the VMAT2-positive compartment of laser scanning confocal images (Nikon A1R), we developed an image analysis method in ImageJ (126). This method identifies regions of interest defined by fluorescence of the VMAT2-mCherry fusion protein and measures fluorescence of the dye within those regions of interest. Standard deviation of pixel intensity of the dye within the mCherry-defined regions of interest provides a measure of the degree of punctate fluorescence. When VMAT2 activity is high, fluorescence within these regions is highly punctate and a high standard deviation of pixel values is calculated. As VMAT2 activity is inhibited by increasing concentrations of TBZ, fluorescence in these regions becomes less punctate and a low standard deviation of pixel values is calculated. From these values, a punctate-diffuse index can be calculated by dividing the standard deviation in each image by the mean of the standard deviations in all control (vehicle treated) images (127). As we reported, this assay measures a 2.5 fold change between positive and negative controls with a Z-factor of 0.62, which indicates the assay is amenable to a high content format (Z-factor of greater than 0.3 for high content imaging) (Table 1).

High content screen assay development

In order to develop this assay for high content analysis, we worked with a ThermoFisher ArrayScan VTI. As shown in Figures 1 and 2, the images captured with the ArrayScan VTI are acquired at a lower optical resolution than the laser scanning confocal images (Figures 1, 2). As reported in our paper, images acquired with the Nikon A1R confocal show a clear punctate pattern of staining in the red channel and a similar pattern of staining in the green
channel with additional staining in the mitochondria (Figure 1) (123). Furthermore, the loss of the punctate pattern of staining can be easily seen in these images (Figure 1). However, images acquired by the ArrayScan VTI, which does not have confocal capability, show a very diffuse pattern of staining in the green channel (Figure 2). Specific localization to the mitochondria and punctate vesicle-like structures are not visible at this resolution.

We developed a protocol in iDev, the ArrayScan image analysis software, to analyze acquired images acquired by the ArrayScan. First, similar to the ImageJ protocol, the iDev protocol identifies objects defined by mCherry fluorescence as primary objects (Figure 3). Very small objects are excluded since these do not correspond to the mCherry-positive extensions where co-localization of the dye and mCherry primarily occurs (Figure 3B). Second, nuclei are identified by staining with Hoescht 33342, a live cell nuclear stain (Figure 3C). Third, the region of interest is defined based on the mCherry positive primary objects by excluding the nuclei and an area of 30 pixels around the nuclei (Figure 3D). Finally, the protocol measures various parameters based on the green fluorescence within the defined region of interest. As shown in Table 1, while this method does detect a 2.3 fold change between positive and negative control wells, the Z factor is 0.01, indicating that there is too much variability in the assay to be useful as a high content screen.

We suspected that this failure was due to the resolution capabilities of the microscope on the ArrayScan compared to those of the Nikon A1R laser scanning confocal. To test this, we imported images acquired on the Nikon A1R into iDev and analyzed them using the iDev protocol, without the exclusion of the area surrounding the nucleus. Analysis of these images by the iDev protocol was successful (Table 1). This method detected a 2.3-fold change, translating to a Z-factor of 0.44. Next, we exported images acquired by the ArrayScan VTI and analyzed them in ImageJ using the protocol reported in our paper (123). This analysis did not detect any differences between vehicle and TBZ treated cells. Finally, we applied deconvolution to ArrayScan VTI images and repeated the analyses; this did not improve the results in ImageJ or iDev (data not shown). Together, the data demonstrate that the resolution of the images acquired on the ArrayScan VTI, not the analysis methods in ImageJ and iDev, is the source of the inability to detect differences in these images.

Unfortunately, the lower resolution images acquired with the ArrayScan VTI are not of a sufficient resolution to be analyzed by either method. Without the submicron resolution of a laser scanning confocal, the microscope is unable to differentiate vesicular fluorescence from surrounding cytosolic fluorescence. The ArrayScan XTI is configured with a higher resolution camera and has a spinning disc confocal module. We tested our assay on this model to determine if the increased resolution of this system was sufficient to allow adaptation of our assay to a high content format. Unfortunately, images acquired on the ArrayScan XTI with confocal produced similar results to those generated on the ArrayScan VTI (data not shown). This is likely because the ArrayScan XTI confocal module is a spinning disc confocal, which does not have the submicron resolution of a laser scanning confocal.
Development of a novel FFN for high throughput screening

As discussed in our paper, FFNs existing at the time of publication were developed for use in slice preparations; they are not taken up by cells in culture or are pH-sensitive (128–130). While pH sensitive dyes are valuable tools for studying vesicular function, it is important to note that while a pH change would affect vesicular function, this additional level of complexity would complicate screens for modulators of VMAT2 function. Though the previously developed FFNs are valuable for real-time spatial analysis of VMAT2 function in brain slices, their characteristics limited their usability for high content assay development. Therefore, we hypothesized in our paper that a non-pH-sensitive FFN that was taken up in cell culture would be the ideal reagent for a high throughput screen for VMAT2 function. Recently, the Sames and Sulzer groups developed a novel FFN (FFN206) that is taken up in cell culture and is not pH-dependent (131). This dye localizes specifically to VMAT2-positive compartments and does not localize to the mitochondria, reducing non-specific noise in the assay. Because the dye localizes only to vesicle-like compartments, observed fluorescence is only due to transport into the vesicle and not mitochondrial or cytosolic staining. This specificity precludes the need for high content imaging and allows the fluorescence to be read by plate reader. The authors demonstrate that FFN206 allows for high throughput analysis with a Z-factor of 0.7–0.8 (131).

Conclusions

Radioactive neurotransmitter uptake in isolated synaptic vesicles has unquestionably enhanced the understanding of VMAT2-mediated transport. However, these assays are not amenable to high throughput screening due to the required high animal expenditure and safety restrictions of radiation usage. Development of the fluorescent high throughput assay overcomes these limitations. Cell lines are a practically unlimited resource, enabling easy replication, generation of dose response curves, and screening of multiple compounds. Such an assay also allows for observation of altered vesicular packaging in an intact cell. This is an important distinction, given that the actions of a compound at VMAT2 in an isolated vesicle are not necessarily the same as its actions at VMAT2 in an intact cell. It is possible that compounds that inhibit VMAT2 in isolated vesicles do not inhibit VMAT2 in an intact cell, if they are impermeable to the plasma membrane. Such a compound would be identified as an inhibitor in a vesicular uptake assay but not in a whole cell assay. Therefore, the whole cell assay more accurately recapitulates the pharmacological action of such a compound in vivo.

Additionally, a whole cell assay enables identification of compounds that indirectly affect vesicular packaging by VMAT2. For example, VMAT2 is known to be regulated by G-protein signaling (132–135). These pathways are unlikely to be intact in isolated vesicles and, thus, compounds that modify VMAT2 function through indirect mechanisms would not be identified. Though they do not fully recapitulate all aspects of a neuron, cell lines contain many intact pathways, allowing for identification of a broader range of compounds that alter vesicular function.

A whole cell fluorescence assay provides a more complete picture of the interplay of plasma membrane and vesicular transporters. Furthermore, fluorescent assays in cell lines can
provide information that is not possible or is cumbersome to gather from traditional radioactive uptake assays, such as time courses and dose response curves. While it will not replace radioactive uptake assays for determination of pharmacokinetic profiles, overall, the low assay cost, low animal usage, the flexibility of this assay and the high content capabilities make an important addition to tools available for studying monoaminergic system. In addition, the ability to assess vesicular uptake in a high throughput format and will allow for faster screening of compounds.

As discussed above, most drugs that target monoaminergic systems act at the plasma membrane transporters (SSRIs, SNRIs) or receptors (pramipexole). However, many of these mechanisms produce compensatory responses, such as changes in receptor density, which reduce long-term treatment efficacy and contribute to adverse effects. Modulation of synaptic vesicle function may be a valuable target for pharmaceutical manipulation since compensatory responses will likely be reduced due to preservation of release and uptake kinetics. The development of novel high throughput assays is an important step towards developing novel therapeutics that target the synaptic vesicle.

References


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Neurochem Int. Author manuscript; available in PMC 2016 September 20.
52. Mariussen E, Fonnum F. The effect of polychlorinated biphenyls on the high affinity uptake of the neurotransmitters, dopamine, serotonin, glutamate and GABA, into rat brain synaptosomes. Toxicology. 2001; 159:11–21. [PubMed: 11250051]


125. Karpowicz RJ, Dunn M, Sulzer D, Sames D. APP+, a Fluorescent Analogue of the Neurotoxin MPP+, Is a Marker of Catecholamine Neurons in Brain Tissue, but Not a Fluorescent False Neurotransmitter. ACS Chemical Neuroscience. 2013

126. Rasband W. (ImageJ. US National Institutes of Health, Bethesda, Maryland, USA.


Highlights

- VMAT1 and VMAT2 transport cytosolic monoamines into synaptic vesicles
- Reduced VMAT function has been linked to neurodegenerative conditions
- Modulation of vesicular function may be beneficial in treating a variety of diseases
- Fluorescent ligands can assess vesicular function in a high throughput format
Figure 1. Images acquired on Nikon A1R laser scanning confocal images.

Images acquired with Nikon A1R laser scanning confocal. The high resolution of this microscope allows for the identification of the punctate localization of the dye in mCherry-positive structures (indicated by arrows) that are localized mostly within extensions from the cell body. Furthermore, the mitochondrial staining pattern is also visible. When VMAT2 function is inhibited by TBZ, the difference in staining is easily observed. Dye can only be seen in the mitochondrial compartment; punctate staining within the mCherry-positive puncta is lost.
Figure 2. Images acquired in high resolution acquisition mode by the ArrayScan VTI without a confocal module

Images acquired with ArrayScan VTI. The mitochondrial pattern of staining of the dye in the cell body region and the punctate staining pattern in the extensions are not visible in these images. Images of the green dye show no difference between treated and untreated cells.
Figure 3. iDev image analysis protocol

(A) Flowchart of iDev protocol steps. (B) Screenshot of primary object identification and validation in channel 1 (mCherry). Primary objects are identified in the red channel. Blue outlines indicate validated objects; orange outlines indicate rejected objects. (C) Screen shot of object identification and validation for channel 2 (Hoescht 33342). Purple outlines indicate validated objects; orange outlines indicate rejected objects. (D) Screen shot of ROI definition. Yellow outlines indicate the area around the nuclei (ROI_B). Cyan outlines indicate the final ROI (ROI_A) defined as the objects validated in Ch1 with ROI_B
excluded. Colocalization analysis is then carried out by measuring channel 3 (dye) within ROI_A.
### Table 1

**Results of ImageJ and iDev analyses**

Top line indicates measurement and fold change between vehicle as positive control and TBZ treatment as negative control. Bottom line shows $Z'$ for the specified images and protocol.

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