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Immunochemical localization of vesicular monoamine transporter 2 (VMAT2) in mouse brain

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Abstract

Vesicular monoamine transporter 2 (VMAT2, SLC18A2) is a transmembrane transporter protein that packages dopamine, serotonin, norepinephrine, and histamine into vesicles in preparation for neurotransmitter release from the presynaptic neuron. VMAT2 function and related vesicle dynamics have been linked to susceptibility to oxidative stress, exogenous toxicants, and Parkinson’s disease. To address a recent depletion of commonly used antibodies to VMAT2, we generated and characterized a novel rabbit polyclonal antibody generated against a 19 amino acid epitope corresponding to an antigenic sequence within the C-terminal tail of mouse VMAT2. We used genetic models of altered VMAT2 expression to demonstrate that the antibody specifically recognizes VMAT2 and localizes to synaptic vesicles. Furthermore, immunohistochemical labeling using this VMAT2 antibody produces immunoreactivity that is consistent with expected VMAT2 regional distribution. We show the distribution of VMAT2 in monoaminergic brain regions of mouse brain, notably the midbrain, striatum, olfactory tubercle, dopaminergic paraventricular nuclei, tuberomammillary nucleus, raphe nucleus, and locus coeruleus. Normal neurotransmitter vesicle dynamics are critical for proper health and functioning of the nervous system, and this well-characterized VMAT2 antibody will be a useful tool in studying neurodegenerative and neuropsychiatric conditions characterized by vesicular dysfunction.
Keywords

VMAT2; Antibody; Monoamine; Immunochemistry; Vesicle; Brain; Localization; Vesicular monoamine transporter 2

1. Introduction

The vesicular monoamine transporter 2 (VMAT2, SLC18A2) is a twelve-transmembrane glycoprotein within the TEXAN (Toxin EXtruding ANtiporter) family of transporters (Eiden et al., 2004). VMAT2 resides on the membrane of secretory vesicles in monoaminergic neurons of the nervous system and in a variety of secretory cells in the gastrointestinal, endocrine, hematopoietic, and immune systems, and is often co-expressed in the periphery with its non-neuronal isoform, VMAT1 (Anlauf et al., 2006, 2004, 2003; Erickson et al., 1992; Henry et al., 1994; Peter et al., 1995a; Schuldiner et al., 1995; Tillinger et al., 2010; Weihe et al., 1994). VMAT2 utilizes an electrochemical gradient maintained by a vesicular H⁺-ATPase to transport one monoamine molecule (dopamine, serotonin, norepinephrine, or histamine) into the highly acidic vesicular lumen in exchange for the efflux of two protons (Chaudhry et al., 2008; Eiden et al., 2004; Erickson et al., 1995; Wimalasena, 2011). The function of VMAT2 is multifold: it prepares neurotransmitters for presynaptic release (Erickson et al., 1992; Henry et al., 1994) and prevents oxidative damage by sequestering deleterious cytosolic monoamines into the vesicle (Alter et al., 2013; Sulzer and Zecca, 2000).

VMAT2 regulates neurotransmitter dynamics and neuronal health (Fon et al., 1997; Wang et al., 1997). Disrupted monoaminergic transmission characterizes a variety of neurodegenerative and neuropsychiatric disorders, such as dystonia, Huntington’s disease, depression, attention deficit hyperactivity disorder, schizophrenia, and addiction (Creese et al., 1996; Eisenberg et al., 1988; Freis, 1954; Hornykiewicz, 1998; Klawans et al., 1972; Ritz et al., 1988; Song et al., 2012). In the case of Parkinson’s Disease (PD), presynaptic monoamine vesicle function is substantially disrupted, and this dysfunction is hypothesized to contribute to neuronal vulnerability in PD pathogenesis (Pifl et al., 2014). In vitro, excess cytosolic dopamine causes intracellular damage via formation of reactive oxygen species in cell cultures (Zhang et al., 2000). In vivo, our laboratory has shown that a mouse model with 95% decreased VMAT2 expression (VMAT2-LO) has reduced ability to sequester deleterious cytosolic dopamine into vesicles and display a number of age-dependent motor and non-motor symptoms associated with PD (Caudle et al., 2007, 2008; Taylor et al., 2011, 2014). Alternately, our laboratory’s mouse model with two-fold VMAT2 protein overexpression (VMAT2-HI) has increased ability to sequester dopamine into vesicles and is protected against dopaminergic degeneration (Lohr et al., 2014, 2015, 2016).

The continuum of VMAT2 gene expression represented by the VMAT2-LO, wildtype (WT), and –HI mice is helpful for confirming the usefulness and specificity of a VMAT2 antibody. Here, we show the use of our polyclonal VMAT2 antibody in a variety of immunochemical assays. The antibody successfully binds to and labels VMAT2, showing specific protein
expression in regions that correspond to monoamine production and release (Ciliax et al., 1995; Fujiwara et al., 1999; Mazzoni et al., 1991; Zhou et al., 1996).

Due to stock depletion, the source of a previously-used effective antibody to VMAT2 is no longer available. Though other groups have had success with other commercially available VMAT2 antibodies (Iritani et al., 2010; Shin et al., 2012; Temple et al., 2016; Zhang et al., 2015), we were unsuccessful in using these antibodies to achieve the specificity and selectivity needed for a battery of immunochemical assays. To address this deficit, we have designed a polyclonal rabbit VMAT2 antibody against a peptide in the C-terminal region of mouse VMAT2. Since creating this antibody, our laboratory has received multiple requests for its use, indicating a need for an effective, well-validated VMAT2 antibody. Here, we use our newly-developed antibody to describe the precise cellular and regional distribution of VMAT2 within the mouse brain.

2. Materials and methods

2.1. VMAT2 antibody production

The C-terminal region of mouse VMAT2 (TQNNVQPYPVGDDEESESD) was conjugated to maleimide activated mcKLH (Thermo Scientific) and sent to Bethyl Laboratories (Montgomery, TX, USA) and Covance Custom Immunology Services (Princeton, NJ, USA) to be injected into two rabbits from each company. Initially, animals were immunized with 500 µg conjugated protein per animal and boosted with 250 µg after 2, 4, and 6 weeks and 125 µg every four weeks thereafter. Sera were collected every other week for 6–12 months and sent back to our laboratory. We optimized the immunochemical use of antisera using VMAT2-WT and –LO brains and VMAT2-transfected HEK cells. Bleeds from one rabbit from Covalence Custom Immunology Services yielded polyclonal anti-VMAT2 serum which passed screening in our immunochemical applications.

2.2. Mice

VMAT2-LO mice were generated by backcrossing the original mixed-background VMAT2-deficient strain (Caudle et al., 2008; Taylor et al., 2014) to Charles River C57BL/6 for four generations using a marker-assisted selection (i.e. “speed congenic”) approach (Lohr et al., 2016). VMAT2-HI mice were generated as previously described (Lohr et al., 2014). Briefly, we used a bacterial artificial chromosome-mediated transgene to insert three additional copies of the murine SLC18A2 (VMAT2) gene, including its endogenous promoter and regulatory elements. These founders were then backcrossed to a Charles River C57BL/6 background. Thus, VMAT2-LO, –WT, and –HI mice share the same genetic background. Mice received food and water ad libitum on a 12:12 light cycle. All procedures were conducted in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at Emory University.

2.3. Differential fractionation

Mouse brains were differentially fractionated using one of three preparations: a crude protein preparation that excludes blood vessels and nuclei, a membrane-associated fraction
containing the presynaptic plasma membrane, and the cytosolic vesicle fraction. In all cases, samples were first homogenized in ice-cold homogenization buffer (320 mM sucrose, 5 mM HEPES, pH 7.4) and protease inhibitors (Sigma, 1:1000) using an immersion homogenizer (Tissue Tearor) for approximately 15 s.

Crude protein preparations were achieved by centrifuging samples at 1150 × g for 5 min, then the resulting supernatant was centrifuged at 18400 × g for 60 min. The resulting pellet was then resuspended in homogenization buffer.

To produce the membrane-associated fraction and the cytosolic vesicle fraction, sample homogenate was centrifuged at 1000 × g for 10 min and the resultant supernatant was centrifuged at 20,000 × g for 20 min. The resultant supernatant was discarded and the pellet was resuspended in homogenization buffer. This resuspended pellet contains isolated synaptosomes. The synaptosomes were osmotically lysed in pure water, then neutralized by addition of HEPES and potassium tartrate (final concentration: 25 mM and 100 mM, respectively). The lysed synaptosomes were centrifuged at 20,000 × g for 20 min. The resultant pellet was suspended in assay buffer (25 mM HEPES, 100 mM potassium tartrate, 100 µM EDTA, 50 µM EGA, pH 7.4). This resuspended pellet is the membrane-associated fraction. The supernatant was centrifuged at 120,000 × g for 2 h and the resultant pellet was resuspended in assay buffer, producing the cytosolic vesicle fraction. Protein content was determined by BCA assay.

2.4. Immunoprecipitation

Immunoprecipitation was performed using the Pierce coimmunoprecipitation kit (Thermo Scientific) according to manufacturer’s protocols. Samples were differentially fractionated into a crude protein preparation, described above. The VMAT2 antibody was cross-linked to agarose beads. Samples were incubated with the antibody-bound columns overnight at 4 °C. Bound protein complexes were eluted the following day and efficacy of immunoprecipitation was determined through immunoblot using the VMAT2 antibody.

2.5. Immunoblot

For the blots in Fig. 1, crude protein preparations from VMAT2-LO, –WT, and –HI striata were prepared as for immunoprecipitation. For the immunoblots shown in Fig. 2, whole brains from VMAT2-WT and –HI animals underwent whole-brain fractionation to yield a membrane-associated fraction and cytosolic vesicle fraction as described above. Samples were not boiled. We used 400 mM dithriothrietol (DTT, Sigma) in NuPage LDS Sample Buffer 4× (Invitrogen) to make 4× loading buffer. We specify these parameters because boiling samples and using non-DTT containing loading buffers appears to destroy the VMAT2-specific epitope. Samples were run on a NuPage 10% bis-tris gel (Life Technologies) and transferred to a PVDF membrane. Nonspecific antibody binding was blocked with a 7.5% milk solution and the membrane was then incubated in primary antibody overnight at 4 ℃. Primary antibodies used were polyclonal rabbit anti-VMAT2 serum (1:10,000), rabbit anti-SV2C (1:5000, developed in our lab, see Stout et al., 2016), mouse anti-alpha-synuclein (1:1000, BD Biosciences 610787), rat anti-DAT (1:1000, Millipore MAB369), rabbit anti-TH (1:1000, Millipore AB152), mouse anti-Rab3 (1:2500,
Transduction Laboratories R35520), mouse anti-amphiphysin (1:10,000, Transduction Laboratories A59420), mouse anti-Bramp2 (1:1000, Transduction Laboratories B67020), mouse anti-complexin 2 (1:500, Transduction Laboratories C60320), mouse anti-rabaptin-5 (1:1000, Transduction Laboratories R78620), mouse anti-rabphilin 3A (1:5000, Transduction Laboratories R44520), mouse anti-rim (1:1000, Transduction Laboratories R69420), mouse anti-sec8 (1:1000, Transduction Laboratories R56420), mouse anti-synapsin IIa (1:5000, Transduction Laboratories S56820), rabbit anti-synaptophysin (1:1000, Millipore AB9272), mouse anti-synaptotagmin I (1:5000, Synaptic Systems 105123), mouse anti-synaptotagmin 2 (1:5000, Synaptic Systems 105123), mouse anti-actin (1:5000, Sigma Aldrich A3853), mouse anti-tubulin (1:5000, Millipore CP06). The following day, the membrane was incubated with the appropriate HRP-linked secondary antibody (1:5000, Jackson ImmunoResearch) for one hour. For preabsorption, a PVDF membrane containing only protein from a VMAT2-LO animal was allowed to soak in 1:10000 VMAT2 antibody for one hour. This antibody solution was then siphoned off and used as primary antibody for other western blot applications, thereby reducing resultant non-specific banding.

2.6. Cell culture and immunofluorescence

We prepared co-cultures from postnatal day 0–2 mice (C57BL/6 mice, The Jackson Laboratory) plating dissociated cells from ventral midbrain, striatum, cortex, and thalamus, each at a density of 20,000 cells/cm² on a monolayer of cortical rat astrocyte cultures (as described in Rayport et al., 1992). Cultures were grown for 2 weeks and fixed with 4% paraformaldehyde for 5 min at RT followed by 10 min 100% methanol at −20 °C. After three 15 min rinses in PBS and a blocking step with 10% normal donkey serum in PBS/0.1% Triton-X for 30 min, dishes were incubated with primary antibodies diluted in PBS/0.1% Triton-X/2% normal donkey serum at 4 °C overnight. The primary antibodies were monoclonal rat anti-DAT (1:500, Millipore MAB369), polyclonal chicken anti-TH (1:1000, Millipore AB9702), and polyclonal rabbit anti-VMAT2 at 1:2000 dilution. Cultures were rinsed again three times for 15 min in PBS and incubated in ALEXA 594/488-conjugated secondary donkey antibodies and DyLight 350-conjugated goat anti-chicken antibody at 1:400 dilution for one hour at RT (Thermofisher), followed by three more rinses in PBS. Images were taken on an Olympus IX81 inverted microscope using 20x and 60x objectives with a Photometrics CoolSNAP HQ2 monochromatic camera controlled by Metamorph software.

2.7. Immunohistochemistry

Immunohistochemistry was performed as previously described (Caudle et al., 2007). Tissue was incubated at 70 °C in Citra (BioGenix) antigen retrieval solution for one hour. Non-specific antibody binding was blocked with a 10% normal goat serum block for one hour at room temperature. Tissue was incubated overnight at 4 °C in polyclonal rabbit anti-VMAT2 serum (1:20,000 or 1:50,000), as indicated. In general, VMAT2-LO tissue was incubated at higher primary antibody concentration in order to increase detection sensitivity in an attempt to visualize any VMAT2 immunoreactive regions. Tissue was then incubated at room
temperature in biotinylated goat anti-rabbit (1:200, Jackson ImmunoResearch) secondary antibody and visualized using a 60-s 3,3′-diaminobenzidine (DAB) reaction. The reaction was terminated with a PBS rinse. All images were acquired with NeuroLucida (MicroBright-Field).

3. Results

3.1. VMAT2 antibody specificity

Immunohistochemical staining revealed strong VMAT2 detection in the striatum, a dopaminergic region. Furthermore, the intensity of VMAT2 staining reflected VMAT2 gene-dose effect across the continuum of VMAT2-expressing transgenic mice. VMAT2-LO mouse striatal slices displayed minimal immunoreactivity for VMAT2. VMAT2-WT and –HI brain slices showed strong, gene-dose dependent immunohistological staining for VMAT2 antibody in the striatum (Fig. 1A). In immunoblot analysis of VMAT2-LO, –WT, and –HI mouse striatal homogenate, the VMAT2-specific bands appeared at approximately 70, 56, and 42 kD, and increased in protein density as gene-dose of VMAT2 increased. The full western blot showed some non-specific band patterns, which was reduced by preabsorbing the VMAT2 antibody on VMAT2-LO tissue. This preabsorption reveals that the gene-dose of VMAT2 protein is maintained across glycosylation weights of VMAT2 (Fig. 1B). Furthermore, the antibody successfully pulled down VMAT2 in an immunoprecipitation assay that was then detected via immunoblot using the VMAT2 antibody (Fig. 1C).

3.2. VMAT2 antibody localizes to synaptic vesicles

By comparison of immunoblots from the membrane-associated fraction and cytoplasmic vesicle fraction of brain homogenate that were probed for a variety of known synaptic vesicle proteins (synaptic vesicle glycoprotein 2C, synaptotagmin 2, synaptophysin, synaptotojanin 1, synopsis II, exocyst complex component 4, rabphilin 3, rabphilin 3A, amphiphysin, and brain form of amphiphysin 2), we confirmed that the VMAT2 antibody localizes to synaptic vesicles. VMAT2-HI mice showed differential expression of VMAT2 in the cytoplasmic vesicle fraction but no differential expression of other synaptic vesicle proteins, indicating that the genetic modification of VMAT2 protein amount did not result in alterations in the expression of these synaptic proteins (Fig. 2).

3.3. VMAT2 expression and localization within a dopaminergic neuron

Co-cultures of dopaminergic midbrain neurons plated with cortical, thalamic, and striatal cells (14 days) were double labeled for VMAT2 and tyrosine hydroxylase (TH) or plasmalemmal dopamine transporter (DAT). VMAT2 label was found exclusively in neurons expressing TH or DAT. The VMAT2 antibody strongly labeled axonal varicosities and axons, while much weaker label was found in cell bodies and dendrites (Fig. 3).

3.4. VMAT2 expression and localization within the dopaminergic system

As expected, VMAT2 antibody staining was reliably observed in dopaminergic cell groups and their terminal fields. In WT mouse brain, VMAT2 antibody staining was observed diffusely through the dorsal and ventral striatum, olfactory tubercle, paraventricular...
dopamine cell populations, and the dopaminergic midbrain (Fig. 3). This staining was negligible in VMAT2-LO mouse tissue, and was darker in VMAT2-HI tissue. This expected differential intensity of VMAT2 antibody staining across gene-dose of VMAT2 demonstrates the specificity of the antibody to the VMAT2 protein. At higher magnification, neurons and processes of the ventral tegmental area (VTA; cell group A10) were immunoreactive for VMAT2 (Fig. 4A). The substantia nigra pars compacta (SNc; cell group A9) displayed a similar pattern of immunoreactivity, with robust labeling of perikarya and a meshwork of neuronal processes (Fig. 4B). VMAT2 immunoreactivity was also observed in the terminal fields of dopaminergic midbrain neurons and was concentrated in neuropil of the dorsal and ventral striatum. Labelling patterns in striatal dopamine terminal regions exhibited compartmental organization with some areas where VMAT2 antibody staining was absent (Fig. 4D, E), likely due to the absence of dopamine terminal markers in the white matter tracts which run through the striatum. Furthermore, VMAT2 antibody displayed staining in paraventricular cell groups (cell groups A11–A15), including sparse perikarya puncta in the more rostral cell groups and preferential granular terminal staining in caudal cell groups along the ventricle (Fig. 4C). These expression patterns are consistent with studies linking VMAT2 to the susceptibility of the dopamine system to dopamine toxicity (Caudle et al., 2007; Reveron et al., 2002), exogenous toxins (Guillot et al., 2008; Lohr et al., 2015; Richardson et al., 2006), and PD-like pathology (Lohr and Miller, 2014; Lohr et al., 2014) (Fig. 5).

3.5. VMAT2 expression and localization within the serotonergic system

VMAT2 antibody stained the serotonergic raphe nucleus in WT tissue. This VMAT2 antibody staining was absent in VMAT2-LO tissue and was more robust in VMAT2-HI tissue. The differential intensity of immunohistochemical staining demonstrates the specificity of the VMAT2 antibody in detecting VMAT2 protein. Higher magnification examination of VMAT2 antibody staining in WT tissue showed dense immunoreactivity in cell bodies and a rich, diffuse meshwork of VMAT2-positive neuronal processes surrounding the dorsal raphe nucleus (Fig. 6A).

3.6. VMAT2 expression and localization within the noradrenergic system

VMAT2 antibody is immunoreactive in the locus coeruleus in VMAT2-WT and –HI tissue, but was negligible in VMAT2-LO brain tissue. VMAT2 antibody displayed very dense cell body staining that was regionally restricted to the triangular locus coeruleus, with a faint meshwork of neuronal processes surrounding these densely labeled cell bodies (Fig. 6B).

3.7. VMAT2 expression and localization within the histaminergic system

Lastly, the VMAT2 antibody stained the histaminergic tuberomammillary nucleus in VMAT2-WT tissue and also exhibited slightly more robust staining in VMAT2-HI brain tissue, but this staining was absent in VMAT2-LO tissue. This difference in staining across VMAT2-altered genotypes lends credence to the specificity of the VMAT2 antibody to VMAT2 protein. The tuberomammillary nucleus shows weak, granular, diffuse staining in WT tissue along the ventral border of the brain, with sparse VMAT2-positive cell bodies (Fig. 6C).
3.8. Additional notable VMAT2 immunohistochemical staining

Furthermore, analysis of VMAT2-WT brains stained with VMAT2 antibody revealed diffuse but notable VMAT2 antibody staining in the medial and lateral habenula, due to the dopaminergic and noradrenergic innervation of the habenula from midbrain neurons and the locus coeruleus, respectively. Additionally, the hippocampus displayed diffuse VMAT2 antibody staining in a laminated pattern in CA1 region of hippocampus, due to the serotonergic and noradrenergic innervation of this region (data not shown).

4. Discussion

These results indicate that our newly developed rabbit polyclonal VMAT2 antibody is specific for the mouse VMAT2 protein and is useful in immunoblot, immunohistochemical, immunocytochemical, and immunoprecipitation studies using homogenates of mouse brain tissue, mouse brain sections fixed via paraformaldehyde, and mouse primary cell culture. The continuum of gene-dose represented by the VMAT2-LO, –WT, and –HI mice—as demonstrated by decreased, normal, and elevated levels, respectively, of VMAT2 mRNA, protein, and function—serves as an excellent series of negative and positive controls for testing the polyclonal VMAT2 antibody (Caudle et al., 2008, 2007; Lohr et al., 2014).

Since the rabbit anti-VMAT2 antibody is polyclonal, the presence of non-specific bands is not surprising. In an effort to minimize non-specific staining, our laboratory attempted to affinity-purify the polyclonal antibody both in-house and through Covance. Unfortunately, affinity purification resulted in a total loss of the VMAT2-specific band. We suspect that this affinity purification strongly bound the VMAT2-specific antibodies such that no specific antibodies were eluted and thus the VMAT2-specific band was lost. However, by preabsorbing the VMAT2 antibody on VMAT2-LO tissue, the non-specific band pattern was greatly reduced. With preabsorption treatment, bands corresponding to varyingly glycosylated VMAT2 are readily apparent, and are consistent with the previously established weights of this protein with post-translational modifications in mouse (Gainetdinov et al., 1998; Richardson et al., 2006; Wang et al., 1997).

Our VMAT2 antibody localizes to the synaptic vesicle, which is consistent with the known cellular localization of VMAT2 (Ericsson et al., 1992; Wimalasena, 2011). In primary dopaminergic cell culture from VMAT2-WT neonates, the VMAT2-antibody colocalizes with dopaminergic markers and permeates the whole dopaminergic neuron, staining the cell body, neuronal processes, and terminal fields. These stained cell bodies and terminal regions are readily apparent via immunohistochemistry. VMAT2 antibody staining was selectively observed in monoaminergic brain regions, including those related to dopamine, serotonin, norepinephrine, and histamine. Indeed, the regional expression pattern of VMAT2 protein in VMAT2-WT and –HI mouse brain aligns with known distributions of markers for dopamine (Ciliax et al., 1995), serotonin (Zhou et al., 1996), norepinephrine (Mazzoni et al., 1991), and histamine (Fujiwara et al., 1999). This labeling is consistent with similar studies showing regional distribution in rat (Cruz-Muros et al., 2008; Erickson et al., 1995; Peter et al., 1995b; Weihe et al., 1994) and primate brain, though primate brains display increased terminal VMAT2 immunohistochemical puncta in the cortex that is not present in rodent models (Erickson et al., 1996a, 1996b; Miller et al., 1999). We observe VMAT2 protein
expression in the same brain regions that the Allen Brain Atlas (freely available at www.brain-map.org) indicates as regions with high VMAT2 mRNA expression, notably in the hypothalamus, midbrain, and brain stem nuclei.

Importantly, VMAT2 gene-dose has been linked to either neurotoxic or neuroprotective effects within each of the monoamine neurotransmitter systems including serotonergic signaling (Alter et al., 2016), noradrenergic degeneration (Taylor et al., 2014), non-motor symptoms of PD (Taylor et al., 2009), and dopamine handling, toxicity, and dopamine-related neurodegeneration (Little et al., 2003; Lohr et al., 2015; Piffl et al., 2014). The significant expression of VMAT2 in dopaminergic regions such as the striatum and midbrain suggests possible sites of action for the previously reported VMAT2-mediated neuroprotection from toxic exposure to pesticides (Richardson et al., 2006), methamphetamine (Guillot et al., 2008; Lohr et al., 2015; Takahashi et al., 1997), and MPTP (Liu et al., 1992; Lohr et al., 2014). The localization of VMAT2 at dopaminergic terminals and the direct mediation of dopamine release by VMAT2 (Lohr et al., 2014) may also provide a mechanism for age-dependent dopaminergic neurodegeneration in VMAT2-LO mice (Caudle et al., 2007). Similarly, VMAT2-LO mice show neurodegeneration of the locus coeruleus (Taylor et al., 2014), a region that also degenerates in PD and shows immunoreactivity to our VMAT2 antibody. This observed reduction in immunoreactivity for VMAT2 in the VMAT2-LO mice is responsible for the disrupted serotonin signaling in these animals (Alter et al., 2016). These effects provide evidence that VMAT2 is broadly important in monoaminergic transmission, with higher gene-dose consistently linked to neuroprotection and lower gene-dose consistently linked to neurodegeneration and neuronal vulnerability.

Overall, the rabbit polyclonal VMAT2 antibody described here will be a useful tool in a variety of immunological methods. This immunochemical characterization of VMAT2 within mouse neurons and circuits will inform the study of monoaminergic vesicular dynamics and associated neurodegenerative and neuropsychiatric diseases.

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Fig. 1.
Molecular specificity of the polyclonal VMAT2 antibody. A. Immunohistochemical staining of VMAT2 is virtually absent in VMAT2-LO brain but is expressed in the striatum of VMAT2-WT and more intensely in VMAT2-HI striatum. Scale bar 1 mm. B. Western blot detection of mouse VMAT2 protein in VMAT2-LO, –WT, and –HI mouse crude synaptosomal striatal homogenate. Left: anti-VMAT2 antibody used at 1:10,000. Right: the same blot was stripped and reprobed with anti-VMAT2 antibody that had been preabsorbed by VMAT2-LO tissue for one hour. VMAT2-specific bands (black arrows) appear at approximately 70, 56, and 42 kD, corresponding with varying VMAT2 glycosylation weights. Bottom: Actin loading control shows no difference in protein amount between lanes. C. Co-immunoprecipitation of VMAT2 from striatal homogenate of WT mice.
Fig. 2.
VMAT2 localizes to synaptic vesicles. Brain homogenate from WT and VMAT2-HI mice was fractionated by differential centrifugation into membrane-associated and cytoplasmic vesicle fractions. VMAT2 expression is highest in the cytoplasmic fraction, in conjunction with other synaptic vesicle proteins, including the synaptic vesicle glycoprotein 2C (SV2C), synaptotagmin 2 (STG2), synaptophysin (SYNP), synaptojanin 1 (SYNJ1), synapsin II (SYN2), exocyst complex component 4 (sec8), rabphilin 3 (rab3), rabphilin 3A (rab3A), amphiphysin (AMPH), and brain form of amphiphysin 2 (BRAMP2). Loading control: beta-tubulin.
Fig. 3. VMAT2 co-localizes with dopaminergic markers in primary cultured TH+ neurons. VMAT2 is expressed in the cell body and neuronal processes of TH+ primary cell culture from VMAT2-WT neonates. Similarly, VMAT2 is co-localizes with DAT in terminal fields primary cell culture. Primary antibodies used at the following dilutions: rat anti-DAT 1:500, chicken anti-TH 1:1000, and rabbit anti-VMAT2 1:2000.
Fig. 4. VMAT2 immunohistochemistry in dopaminergic regions of mouse brain. VMAT2-LO (A, B, C) mice show negligible VMAT2 immunohistochemical staining in dopaminergic brain regions. In both VMAT2-WT and –HI mice, VMAT2 protein is expressed in dopaminergic brain regions. Polyclonal rabbit anti-VMAT2 was used at 1:50,000 on VMAT2-WT and –HI mice, 1:20,000 for VMAT-2 LO tissue. Scale bar 1 mm.
Fig. 5.
VMAT2 immunohistochemistry in dopaminergic cell groups and terminal regions in mouse brain. VMAT2 antibody binds the cell bodies of dopamine neurons in the ventral tegmental area (A), the substantia nigra pars compacta (B), the A14 nucleus (C) and dopaminergic terminal regions in the dorsolateral striatum (D) and ventral striatum (E). Polyclonal rabbit anti-VMAT2 was used at 1:50,000 on VMAT2-WT tissue. Scale bar 100 µm.
Fig. 6.
VMAT2 immunohistochemistry in a serotonergic, noradrenergic and histaminergic nuclei of mouse brain. VMAT2 antibody staining is negligible in VMAT2-LO tissue. In VMAT2-WT and –HI tissue, VMAT2 antibody stains the serotonergic raphe nucleus (A), serotonergic raphe nucleus (B), and histaminergic tuberomammillary nucleus (C). The cell bodies of each of these structures are readily apparent at higher magnification in WT tissue (far right). Polyclonal rabbit anti-VMAT2 was used at 1:50,000 on VMAT2-WT and –HI mice, 1:20,000 for VMAT-2 LO tissue.