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Supplemental Data

Interplay Between Cytosolic Dopamine, Calcium and α-Synuclein Causes Selective Death of Substantia Nigra Neurons

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Supplementary Table 1. Effect of various metabolic inhibitors on carbon fiber electrode sensitivity for DA

Recordings were conducted in 2 ml of Tyrode's saline in the presence of an inhibitor. After ~10 seconds, another 2 ml of the same saline including 10 μM DA was added. The amplitudes of the DA oxidation current on cyclic voltammograms were normalized to those produced by 5 μM DA in the absence of drugs. Data are mean ± SEM from 3-6 measurements.

Compound	μM	DA signal, % of control
L-DOPA	100	79 ± 3
Reserpine	2	75 ± 3
Pargyline	10	86 ± 2
Benserazide	2	93 ± 6
NSD-1015	500	0 ± 0*
Cocaine	10	97 ± 2
Nomifensine	5	81 ± 4
Methamphetamine	5	111 ± 8
Methamphetamine	50	81 ± 1
Cadmium chloride	30	123 ± 13
BAPTA-AM	10	96 ± 9
BAPTA-AM	100	72 ± 2
Nimodipine	10	103 ± 1

* - $p < 0.001$ vs. DA only by one-way ANOVA followed by Tukey's post-hoc test.

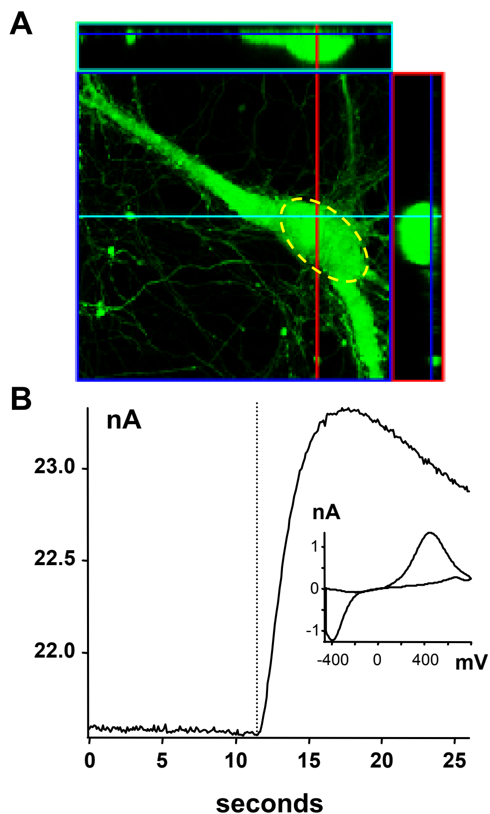

Figure S1. IPE measurements from TH-GFP neurons.

A. Pseudo-3D image of a live ventral midbrain GFP-positive neuron from TH-GFP mice. Insets on the top and right show vertical sections of the neuron at planes indicated by the blue and red lines, respectively. An approximation of neuronal soma is shown as yellow oval.

B. Representative cyclic voltammetry trace from GFP-positive neuron treated with 100 µM L-DOPA for 1h; the current was sampled at 450 mV. Cell membrane rupture is indicated by the dotted line. The inset shows a typical catecholamine voltammogram taken at the maximum of DA oxidation wave.

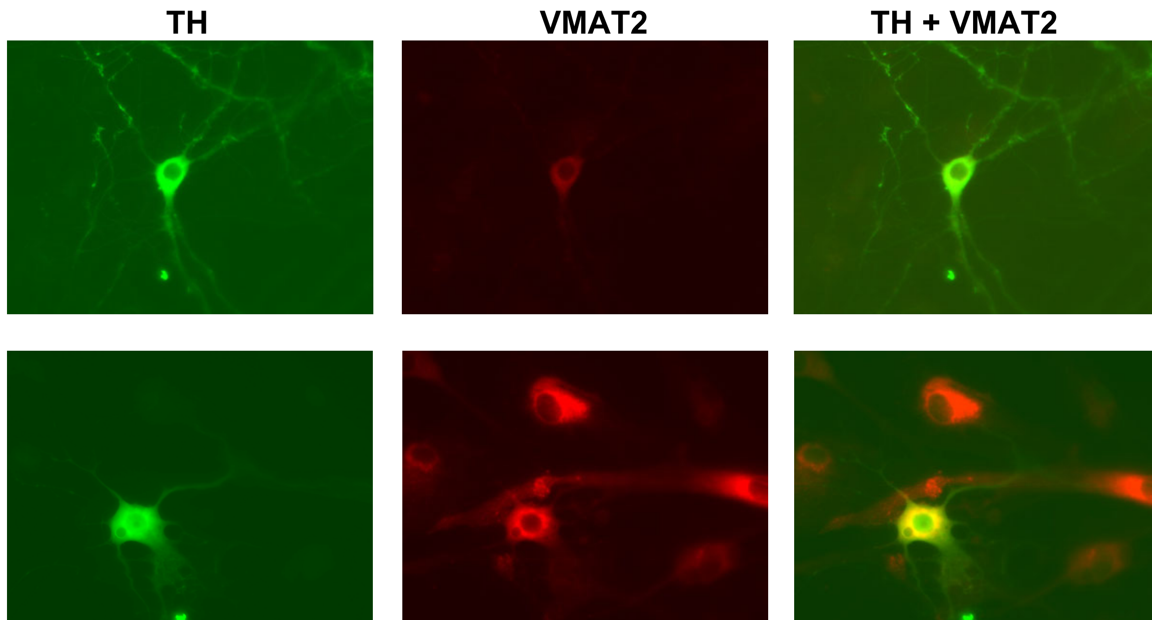

Figure S2. Overexpression of VMAT2 in ventral midbrain neurons.

Representative images of mouse VM neurons (7-day post-plating) either untreated (upper row) or infected with rVMAT (lower row) and double-stained for TH (green) and VMAT2 (red). Overlaid fluorescent images show complete colocalization of the two fluorescent markers in untreated neurons. Cultures exposed to rVMAT2 show increased VMAT2 immunoflourescence in the cell bodies of TH+ neurons, as well as an expression of the protein in non-dopaminergic cells.

As previously published (Krantz et al., 1997), expression of rVMAT2 was confirmed by immunostaining for both VMAT2 and the HA epitope placed in the lumenal loop between TMDs 1 and 2 that does not interfere with transporter activity or its subcellular localization. Overexpression of VMAT2 in VM neurons increased the overall transporter levels measured by western blot, and enhanced the depolarization-evoked release of DA and the total intracellular DA by ~2-fold relative to control cells as assessed by HPLC-EC (Pothos et al., 2000). Transfection efficiency reached 80-90% with expression of VMAT2 protein observed in both dopaminergic and non-dopaminergic neurons.

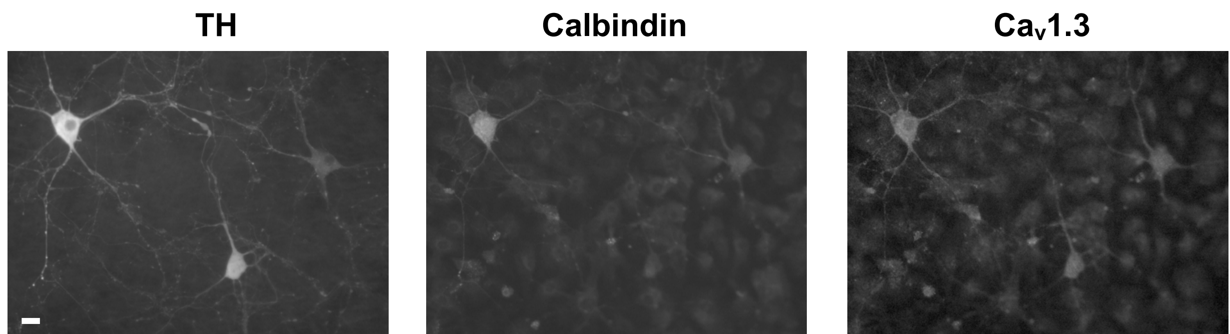

Figure S3. Immunocytochemistry of $Ca_v1.3$ channel in SN and VTA neurons.

Mouse VM neurons were fixed and stained for TH, calbindin and the α1D subunit of the L-type Ca^{2+} channels, which is uniquely present in the $Ca_v1.3$ channel. No difference in the $Ca_v1.3$ staining was observed between SN (calbindin-) and VTA (calbindin+) DA neurons. Scale bar is 20 microns.

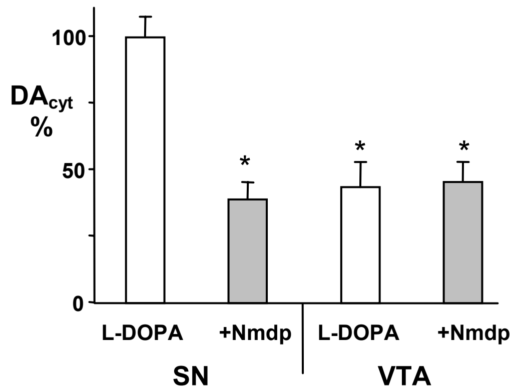

Figure S4. Comparison of DA_{cyt} in SN and VTA neurons before and after nimodipine treatment.

SN and VTA neurons were pre-treated with 10 μM nimodipine for 1h, and then exposed to 100 μM L-DOPA for 1h. DA_{cyt} concentrations were normalized to the levels in SN neurons treated with L-DOPA only. * - $p<0.05$ vs. SN with L-DOPA only by one-way ANOVA.

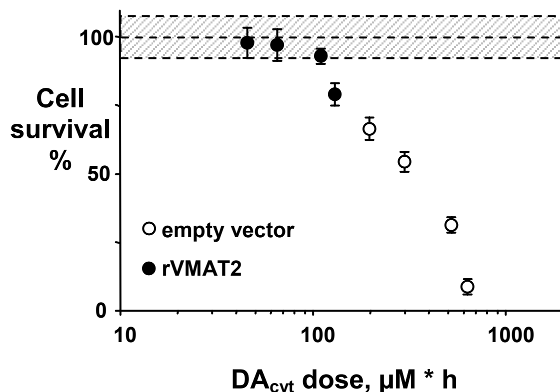

Figure S5. Dependence of TH+ neurons survival on DA_{cyt} dose in VM cultures treated with rVMAT.

DA_{cyt} dose was calculated as described in Figure 7A legend, using IPE data from mouse VM neurons transfected with rVMAT2 or an empty vector (Figure 4A). Cell survival was determined in rat VM cultures transfected with empty vector or rVMAT2 and treated with 50, 100, 500 and 1000 μM L-DOPA for 7 days. Dotted lines and shadowed boxes represent mean ± SEM in untreated cells.