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Roles of VGLUT2 and Dopamine/Glutamate Co-Transmission in Selective Vulnerability to Dopamine Neurodegeneration

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Abstract

Growing evidence has established that a subset of dopamine (DA) neurons co-release glutamate and express vesicular glutamate transporter 2 (VGLUT2). VGLUT2 expression in DA neurons plays a key role in selective vulnerability to DA neurodegeneration in Parkinson's Disease (PD). In this review, we summarize recent findings on impacts of VGLUT2 expression and glutamate co-release from DA neurons on selective DA neuron vulnerability. We present evidence that DA neuron VGLUT2 expression may be neuroprotective, boosting DA neuron resilience in the context of ongoing neurodegenerative processes in PD. We highlight genetic and pesticide models of PD that have provided mechanistic insights into selective DA neuron vulnerability. Finally, we discuss potential neuroprotective mechanisms, focusing on roles of VGLUT2 and glutamate in promoting mitochondrial health, and diminishing oxidative stress and excitotoxicity. Elucidating these mechanisms may ultimately lead to more effective treatments to boost DA neuron resilience that can slow or even prevent DA neurodegeneration.

Graphical Abstract

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Author Contributions

SAB and ZF conceived the idea and designed the manuscript. SAB, MQEO, SHB, CDM, VPR, and SAR wrote the manuscript. SAB, MQEO, and ZF created figures and performed editing. All authors approved the final manuscript version.

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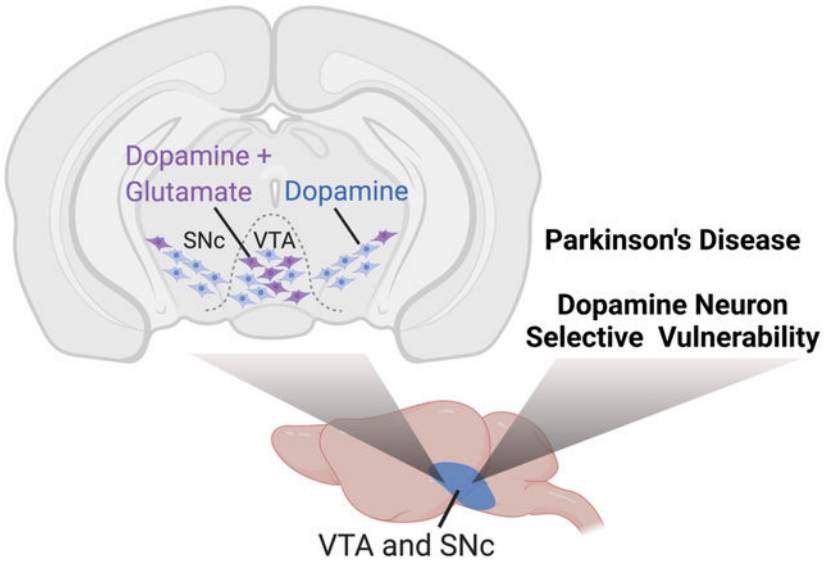

Keywords

Dopamine; glutamate; co-release; VGLUT2; Parkinson's Disease; neurodegeneration

Overview

Following early evidence of glutamate transmission in cultured dopamine (DA) neurons[1, 2], subsequent studies verified that a minority of DA neurons express vesicular glutamate transporter 2 (VGLUT2) and co-release glutamate. This phenomenon is conserved across evolution, having been observed in flies, rodents, non-human primates, and humans[3–5]. The majority of midbrain DA/glutamate neurons localize to the medial ventral tegmental area (VTA) and project to the medial shell of the nucleus accumbens (NAc), while a smaller DA/glutamate neuron population in the lateral substantia nigra *pars compacta* (SNc) projects to the tail of the striatum[6, 7]. Remarkably, almost all DA neurons express VGLUT2 during development, only to repress VGLUT2 in adulthood[8, 9]. However, VGLUT2 expression is increased in DA neurons, and associated with DA neuron survival, after exposure to insults[10–12]. This raises the question of what roles VGLUT2 expression and glutamate co-transmission may play in selective DA neuron vulnerability in neurodegenerative diseases including Parkinson's Disease (PD). Here, we summarize findings on DA neuron VGLUT2 and its impact on selective DA neuron vulnerability, with a focus on pesticide and genetic models of PD. We also explore potential mechanisms of DA neuroprotection conferred by VGLUT2 expression.

VGLUT2 and Selective DA Neuron Vulnerability in PD

Even before the discovery of DA/glutamate neurons, changes in glutamatergic neurotransmission within DAergic brain areas were observed in response to DA neuron loss where treatment with DA neurotoxin 6-hydroxydopamine (6OHDA) increases striatal glutamate release[13]. Importantly, the proportion of VGLUT2-expressing DA neurons in the

VTA and their projections to the NAc increases after single exposures to DA neurotoxins (*e.g.*, 6OHDA, MPTP) at early postnatal periods and in adulthood[2, 11, 14, 15] or to chronic α-synuclein exposures in mouse PD models[12]. This is accompanied by upregulated VGLUT2 expression in surviving DA neurons[9, 12]. Conversely, VGLUT2 cKO in DA neurons exacerbates DA neurodegeneration in response to 6OHDA and MPTP[9, 11, 16], and leads to fewer striatal connections after a 6OHDA-induced lesion[16]; VGLUT2 cKO mice also show impairments in DA-dependent basal and psychostimulant-induced locomotion after MPTP[3, 11, 17]. In humans, elevated striatal VGLUT2 expression was discovered in brains of PD patients[18]. Consistent with this, VGLUT2-expressing DA neurons are enriched in the SNc of PD patients versus controls[12], further suggesting that VGLUT2 boosts DA neuron resilience in PD. Overall, these findings suggest roles for VGLUT2 in selective DA neuron vulnerability.

Males are likelier to develop PD versus females[19] and age remains the greatest risk factor in PD[20]. In *Drosophila*, the *Drosophila* ortholog of VGLUT, dVGLUT, mediates sex differences in vulnerability to DA neurodegeneration[5]. These studies showed that DA neuron dVGLUT expression increases with age, and RNA interference (RNAi)-mediated knockdown of dVGLUT in DA neurons increases age-related DA neurodegeneration[5]. Further, there are evolutionarily conserved sex differences in DA neuron VGLUT expression with females exhibiting greater DA neuron VGLUT expression than males in *Drosophila*, rats, and humans[5]. Consequently, DA neuron VGLUT is implicated in age- and sex-related differences in DA neurodegeneration. Nevertheless, questions remain concerning the translatability of these age and sex differences from animal models to clinical PD. Recent work showing greater resilience of VGLUT2-expressing SNc DA neurons was only conducted on male subjects[12]. Therefore, investigating DA neuron VGLUT2 expression in female PD patients is a critical next step and may provide a novel mechanism by which females are more protected from DA neurodegeneration in PD.

Though regulation of *endogenous* VGLUT2 expression appears neuroprotective for DA neurons, *heterologous* VGLUT2 overexpression in DA neurons offers conflicting results. In mice, DA neuron-specific VGLUT2 overexpression was selectively neuroprotective in one study and neurotoxic in another[9, 11]. Recent work demonstrated that the lowest levels of heterologous VGLUT2 overexpression are closest to physiological levels of upregulation which boost DA neuron resilience to MPTP[5]. In contrast, higher levels of VGLUT2 overexpression increase selective vulnerability of midbrain DA neurons[5]. Since most DA neurons only transiently express VGLUT2 during development before losing VGLUT2 expression by adulthood[9], these mature DA neurons may not be equipped for sustained VGLUT2 overexpression. This suggests DA neuron VGLUT2 expression must be finely tuned through endogenous regulatory mechanisms to enhance DA neuron resilience. Circumventing such mechanisms by changing VGLUT2 expression at either extreme impacts resilience[5]. Thus, insufficient VGLUT upregulation may not boost neuronal resilience enough to withstand cell stress, whereas too much VGLUT2 expression diminishes resilience.

DA Neuron VGLUT2 and Selectivity Vulnerability in Pesticide Models of PD

Pesticide exposure can lead to DA neurodegeneration across several animal models and to PD in humans[21–23]. Pesticide models induce progressive DA neuron loss and reproduce key features of PD neuropathology that are not observed with neurotoxicants like 6OHDA or MPTP, including accumulation of intracellular α-synuclein and polyubiquitin aggregates in DA neurons[22–27]. We will focus on paraquat and rotenone, pesticides that cause selective DA neurodegeneration and differ mechanistically.

Paraquat, one of the most used pesticides globally, is associated with increased PD risk[22]. Occupational exposure to paraquat doubles PD risk and even indirect exposures boost PD risk[28]. In mice, paraquat causes SNc DA neurodegeneration, analogous to the pattern of cell loss in early PD[29]. Paraquat achieves these effects via generation of reactive oxygen species (ROS) that impair mitochondrial function and ultimately lead to DA neurodegeneration[30] (Figure 1A).

Rotenone is associated with sporadic PD and/or amplifying pre-existing PD risk[25, 31]. Rotenone exposure causes selective degeneration of nigral DA neurons through oxidative stress via direct inhibition of Complex I of the mitochondrial electron transport chain[25] (Figure 1B). Chronic, systemic rotenone administration in rats recapitulates hallmark PD pathology, causing progressive deficits in neuroinflammatory and autophagy-lysosomal pathways, as well as accumulation of α-synuclein and polyubiquitin aggregates in DA neurons[23, 25]. Moreover, as in PD, VTA DA neurons are relatively spared after rotenone versus the SNc[25].

Though the mechanisms underlying selective vulnerability of midbrain DA neurons remain unclear, we can glean insights using pesticide models. Vesicular transporters may offer mechanistic clues. Vesicular monoamine transporter 2 (VMAT2) facilitates DA loading into vesicles. In this process, VMAT2 sequesters DA into synaptic vesicles, diminishing cytoplasmic DA available for degradation into products that raise toxic ROS to damage mitochondria and injure DA neurons via oxidative stress[32, 33]. Besides VMAT2, VGLUT2 expression in DA neurons is also protective against rotenone[10], consistent with VGLUT2's ability to boost resilience in DA neurons across different PD models.

Mechanisms of Protection of VGLUT2-Expressing DA Neurons

Since PD is a pleiotropic, multifactorial illness based on complex gene-environment interactions, familial PD cases caused by mutations in specific genes have provided vital insights into PD pathogenesis and mechanisms of selective DA neuron vulnerability. In addition to α-synuclein, there is substantial evidence of PD associations with *PINK1* and *Parkin* genes which encode a serine/threonine ubiquitin kinase and an E3 ubiquitin ligase, respectively[37, 38]. PINK1 and Parkin proteins have major functions in mitophagy, a form of autophagy involving the selective trafficking of mitochondria for degradation[37, 39] (Figure 2A). Impairment or disruption of PINK1/Parkin functions contribute to PD pathogenesis[39, 40]. Consistent with this, cells with decreased PINK1 display reduced mitochondrial membrane potential, respiratory impairments, mitochondrial

calcium overload, and heightened ROS production. PINK1 mutants that are no longer trafficked to mitochondria cannot form functional Parkin/PINK1 complexes, causing accumulation of damaged, dysfunctional mitochondria[41, 42]. This boosts mitochondrial ROS production as well as upregulates TH to increase cytoplasmic DA levels available for oxidation into DA quinones. These effects collectively contribute to ROS generation and oxidative stress[43], ultimately leading to DA cell death in PD (Figure 2B).

An imbalance between ROS production and antioxidant activity leads to mitochondrial dysfunction and has been cited as potential cause of PD. PD-linked DJ-1 (encoded by *PARK7*) has a neuroprotective function by maintaining or restoring the balance between mitochondrial ROS generation and antioxidant activity. DJ-1 achieves this as an antioxidant scavenger or redox sensor, as well as a redox-sensitive chaperone that inhibits α-synuclein accumulation[46, 47]. Indeed, mice deficient in DJ-1 exhibit a fragmented mitochondrial phenotype which causes oxidative stress in DA neurons and can be rescued by PINK1 and Parkin[46].

Studies in flies, rodents, and human brain suggest VGLUT (VGLUT2 in mammals and dVGLUT in *Drosophila*) expression in DA neurons mediates conserved neuroadaptive responses to insults which may play a role in selective vulnerability in PD[5, 10]. We posit that VGLUT's ability to diminish cytotoxic intracellular ROS and maintain mitochondrial health is integral to its neuroprotective properties (Figure 3A). Several lines of evidence link DA neuron VGLUT to mitochondrial function: 1) Recent electron microscopy studies revealed that VGLUT2^{+} synaptic vesicles in nerve terminals projecting to the NAc are in closer proximity to mitochondria versus other vesicle populations[48]. 2) Mitochondrial availability is linked to synaptic dVGLUT levels[49]. 3) VGLUT2 enhances DA sequestration into synaptic vesicles during periods of increased neuronal activity[3, 50]. This diminishes cytoplasmic DA available for degradation into products that raise toxic ROS and produce mitochondrial dysfunction. Indeed, VGLUT2-expressing DA neurons are less vulnerable to rotenone-induced cell loss[10]. Finally, VGLUT2 may be an important modulator of axon arborization in DA neurons, suggesting that VGLUT2 may help DA neurons re-form axonal branching in response to stressors. VGLUT2 cKO in DA neurons impairs axonal reinnervation in the striatum in response to 6OHDA[16]. This relationship between VGLUT2 expression and axonal arborization may be regulated by glial-derived neurotrophic factor (GDNF)[16] and brain-derived neurotrophic factor and tropomyosin-receptor kinase B (BDNF/TrkB) signaling[11].

The greater resilience of VGLUT2-expressing DA neurons may also stem from glutamate-dependent mechanisms (Figures 3B, 3C). VGLUT2 limits glutamate availability by vesicular glutamate sequestration after insults to protect neurons from glutamate excitotoxicity[51] (Figure 3B). Additionally, midbrain DA neurons express the glutamate transporter EAAT3. EAAT3 transports glutamate into the cell from the extracellular space and is dynamically trafficked at the plasma membrane to modulate synaptic glutamate signaling[52]. This suggests EAAT3 function in DA/glutamate neurons may boost resilience by increasing reuptake of synaptic glutamate as protection against glutamate-mediated excitotoxicity (Figure 3B). Moreover, glutamate serves as an anaplerotic energy source as well as a source of the antioxidant glutathione. Glutamate can be converted to α-ketoglutarate either

directly in neurons or in nearby astrocytes to continue fueling the TCA cycle to maintain ATP synthesis during stress[53, 54]. Also, as glutathione synthesis depends on glutamate[55], glutamate availability helps replenish glutathione to protect DA neurons from oxidative stress (Figure 3C). The combination of these mechanisms may explain the greater resiliency of DA neurons that co-release glutamate.

Finally, while the neuroprotective mechanisms proposed above are cell autonomous, VGLUT2-expressing DA neurons may also be protected through non-cell autonomous mechanisms. Recent work has begun to investigate this by focusing on DA/glutamate neuron projections to cholinergic interneurons[56, 57]. Glutamate co-release from VGLUT2-expressing DA neurons drives cholinergic interneuron burst-firing, causing acetylcholine-mediated stimulation of nicotinic acetylcholine receptors on the terminals of the DA/glutamate neurons to cause further DA/glutamate co-release[7]. Such a positive feedback loop may enable VGLUT2-expressing DA neurons to maintain their function and ultimately enable survival during cell stress.

Concluding Remarks and Future Perspectives

Many questions remain concerning the physiological relevance of DA/glutamate co-transmission and its relevance to healthy and disease states. Indeed, though almost all DA neurons express VGLUT2 as they develop, the regulatory mechanisms by which most DA neurons switch off VGLUT2 expression post-development or how these cells re-activate VGLUT2 expression in response to insults remain open questions. Increasing evidence shows that DA neuron VGLUT2 may act as a determinant of selective DA neuron vulnerability in PD. While we propose potential mechanisms by which VGLUT2 and glutamate modulate protection of DA neurons, future work is required to clarify these mechanisms. Elucidating these mechanisms may ultimately lead to new, more effective treatments for neuropsychiatric disorders including PD.

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Abbreviations

α-KG	α-ketoglutarate
cKO	conditional knockout
DA	dopamine
DAT	dopamine transporter
EAAT3	excitatory amino acid transporter 3

ETC	electron transport chain
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NAc	nucleus accumbens
PD	Parkinson's disease
PQ	paraquat
RNAi	RNA interference
SNc	substantia nigra *pars compacta*
TH	tyrosine hydroxylase
VMAT2	vesicular monoamine transporter 2
VTA	ventral tegmental area
6OHDA	6-hydroxydopamine
VGLUT2	vesicular glutamate transporter 2

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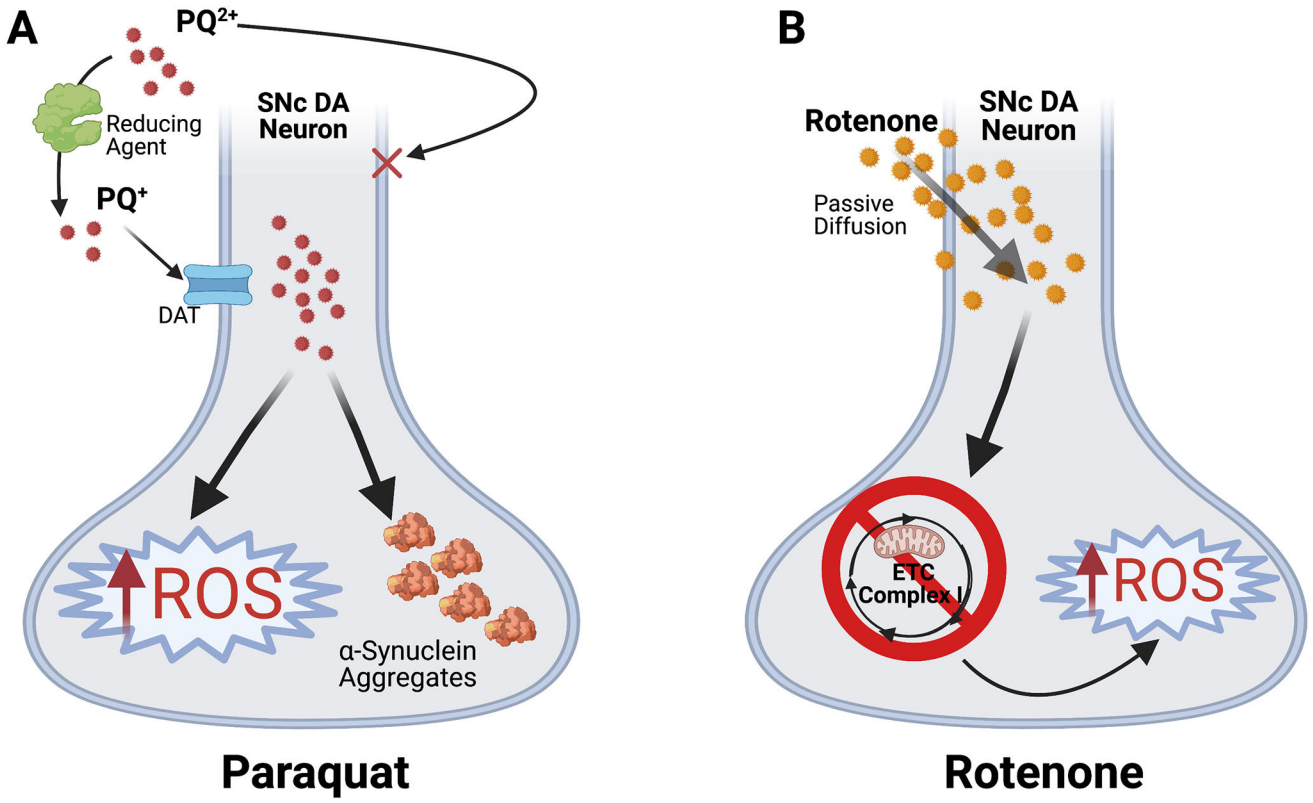

Figure 1. Mechanisms of pesticide-induced SNc dopamine neurodegeneration.

(A) Paraquat (PQ) enters the organism in its native divalent cation state, PQ^{2+}, which cannot cross the plasma membrane. However, conversion to PQ^{+} by a reducing agent enables PQ^{+} to selectively enter DA neurons via the dopamine transporter (DAT)[34]. PQ^{+} accumulates in neurons where it induces increased ROS generation and α-synuclein aggregation[35]. Resulting oxidative stress contributes to SNc DA neurodegeneration. **(B)** Rotenone is lipophilic and freely crosses the membrane via passive diffusion[36]. Rotenone then induces ROS buildup and neurotoxic oxidative stress via direct inhibition of Complex I of the mitochondrial electron transport chain (ETC)[25].

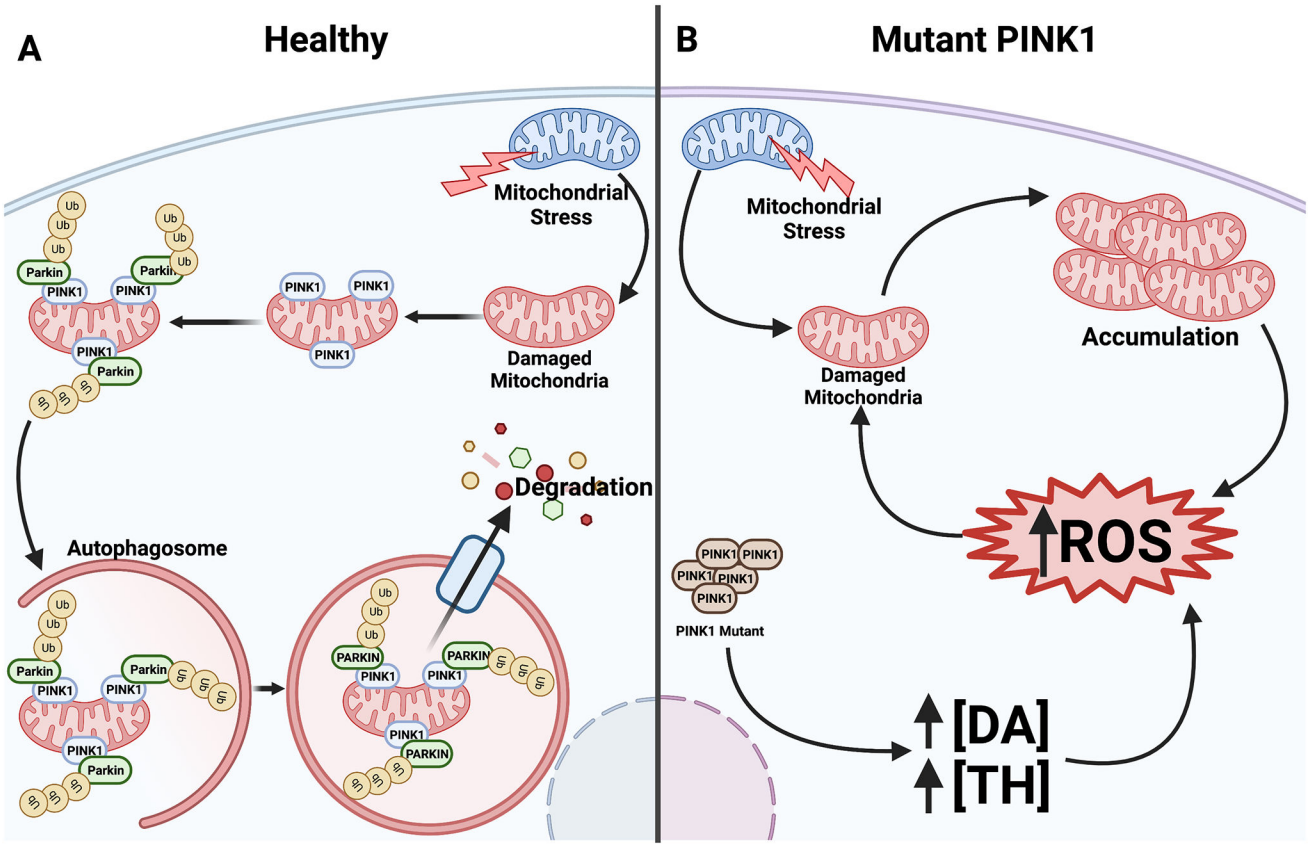

Figure 2. Roles of Parkin/PINK1 in mitochondrial quality control and effects of PINK1 mutants in DA neurons.

(A) PINK1 accumulates on damaged mitochondria, which signals the E3 ubiquitin ligase PARKIN to bind[44]. PARKIN requires PINK1 to activate it[45], resulting in ubiquitination which targets the mitochondria for degradation by autophagosome-mediated mitophagy. **(B)** PINK1 mutants have difficulty localizing to and binding damaged mitochondria. In the absence of functional Parkin/PINK1 complexes to signal mitophagy, damaged mitochondria accumulate which leads to ROS buildup. Mutant PINK1 also upregulates TH expression to increase cytoplasmic DA. Resulting elevation in ROS generation further boosts oxidative stress to cause DA neurodegeneration.

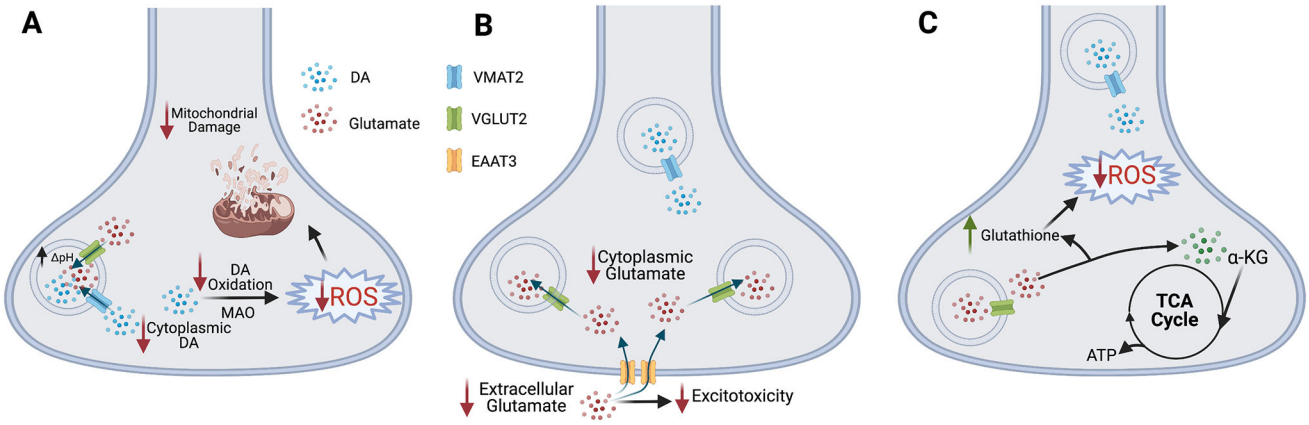

Figure 3. Potential mechanisms of VGLUT2-mediated protection from neurotoxicity.

(A) VGLUT2 enhances DA loading into synaptic vesicles via VMAT2 by increasing the vesicular pH gradient (ΔpH)[50]. This decreases cytoplasmic DA levels, making less free DA available to generate neurotoxic ROS that damage mitochondria. **(B)** VGLUT2-mediated glutamate sequestration lowers cytoplasmic glutamate to diminish glutamatergic excitotoxicity. VGLUT2-expressing DA neurons also express EAAT3, which removes glutamate from the extracellular space, preventing glutamate-mediated excitotoxicity. **(C)** Glutamate can be converted to α-ketoglutarate (α-KG) to feed into the TCA cycle and maintain mitochondrial ATP production. Glutamate also replenishes glutathione to reduce ROS accumulation and protect DA neurons.