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REVIEW ARTICLE

Dopamine compartmentalization, selective dopaminergic vulnerabilities in Parkinson's disease and therapeutic opportunities

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Abstract

Progressive depletion of selected dopamine neurons is central to much Parkinson's disease (PD) disability. Although symptomatic treatments can ameliorate the disabilities that this neuronal depletion causes, no current strategy is documented to slow these losses. There is substantial evidence that dopamine in intracytoplasmic/extravesicular neuronal compartments can be toxic. Here, I review evidence that supports roles for dopamine compartmentalization, mediated largely by serial actions of plasma membrane SLC6A3/DAT and vesicular SLC18A2/VMAT2 transporters, in the selective patterns of dopamine neuronal loss found in PD brains. This compartmentalization hypothesis for the dopamine cell type specificity of PD lesions nominates available drugs for amelioration of damage arising from miscompartmentalized dopamine and raises cautions in using other drugs.

Lost integrity of dopamine neurons whose cell bodies lie in the ventral midbrain is the clearest pathological correlate of much of the rigidity and bradykinesia that provide significant morbidity in Parkinson's disease (PD).[1] These neurons also suffer losses with aging, in ways that comport with the powerful influence of age as a PD risk factor.[2–6] Effective symptomatic therapies that replace lost dopaminergic tone can offset some of the consequences of dopaminergic neuronal losses.[7] Other neuronal groups are also at risk to varying extents in both aging and PD.[8] Nevertheless, slowing losses in the integrity of ventral midbrain dopaminergic neurons would be likely to significantly slow progression of key PD symptoms and aging effects on motor abilities.[7] There is no satisfactory current drug therapy that prevents progression of the losses of these key dopamine neurons in aging or PD. Optimally preserving the health of important ventral midbrain dopamine neurons provides a major potential focus for efforts to understand and treat dopaminergic declines in PD and aging.

We and others have long been impressed by several features of dopamine and the biology of the dopaminergic neurons that modulate locomotor activities and mood.[9] Dopamine can be both a neurotransmitter and a neurotoxin. Dopamine can exert toxicities in several ways, including (a) accelerating redox processes[10,11] (b) forming protein adducts in some cellular environments[12–14] and (c) altering synuclein aggregation.[15] In the low pH of synaptic vesicles, into which it is pumped by the synaptic vesicular monoamine transporter (SLC18A2/VMAT2), dopamine is unlikely to exert such toxic activities (Fig. 1). In the intracellular/extravesicular cytosolic compartment into which it is pumped by the plasma membrane dopamine transporter (SLC6A3/DAT), dopamine can exert substantial damage. Aspects of dopamine synthesis and metabolism are also localized in this compartment.[16] Inhibitors of monoamine oxidase that

reduce dopamine redox damage in this intracellular/extravesicular cytosolic compartment can modestly reduce PD progression in some, though not all, studies.[17–19] Evidence reviewed here, including the consequences of altering dopamine compartmentalization via amphetamine or cocaine actions on compartmentalizing transporters, now provides major support for the dopamine compartmentalization hypothesis (see below).

SLC6A3/DAT (dopamine transporter) is a member of the 12-transmembrane domain, sodium- and chloride-dependent neurotransmitter transporter family that is largely expressed in the plasma membranes of dopaminergic neurons.[20–22] This protein's activities contribute dramatically to the regulation of the spatial spread and temporal persistence of signals that arise when dopamine is released by dopamine neurons. SLC6A3/DAT also mediates the selective dopaminergic cellular accumulation of some of the most studied dopamine-selective neurotoxins.[9,23] Although the degree to which these dopaminergic neurotoxins mimic PD pathophysiology is questioned,[24] experimental overexpression of SLC6A3/DAT in nonneuronal or GABA/non-dopamine neurons allows MPP^+ and/or dopamine to kill previously resistant cells that now acquire the ability to concentrate these toxins in cytoplasmic/extravesicular compartments.[25,26] While SLC6A3/DAT is a principal site of action for cocaine and other rewarding psychostimulants,[27] not all SLC6A3/DAT blockers cause cocaine-like euphoria or display sizable abuse liability (see below).

Movement from the intracytoplasmic/extravesicular compartment into synaptic vesicles uses the principal brain synaptic vesicular monoamine transporter, SLC18A2/VMAT2. SLC18A2/VMAT2 encodes another 12-transmembrane domain transporter gene family member that uses proton gradients to pump monoamines into synaptic vesicles, along whose membranes SLC18A2/VMAT2 is largely localized.[28] This transporter's ability to sequester MPP^+ and dopamine into synaptic vesicles is manifest by greater MPP^+ and dopamine toxicities when SLC18A2/VMAT2 expression levels are reduced.[29,30] Experimental overexpression of SLC18A2/VMAT2 in nonneuronal cells confers MPP^+ resistance onto cells that acquire the ability to detoxify by concentrating MPP^+ into vesicles.[31] I have hypothesized that intracellular/extravesicular concentrations of dopamine, regulated chiefly by serial actions of SLC6A3/DAT and SLC18A2/VMAT2, make large contributions to the selective dopaminergic damage in Parkinsonism and in normal aging.[9] Below, I summarize and update evidence that now supports this testable and treatable dopamine miscompartmentalization mechanism for cell-specific contributions to PD pathogenesis.

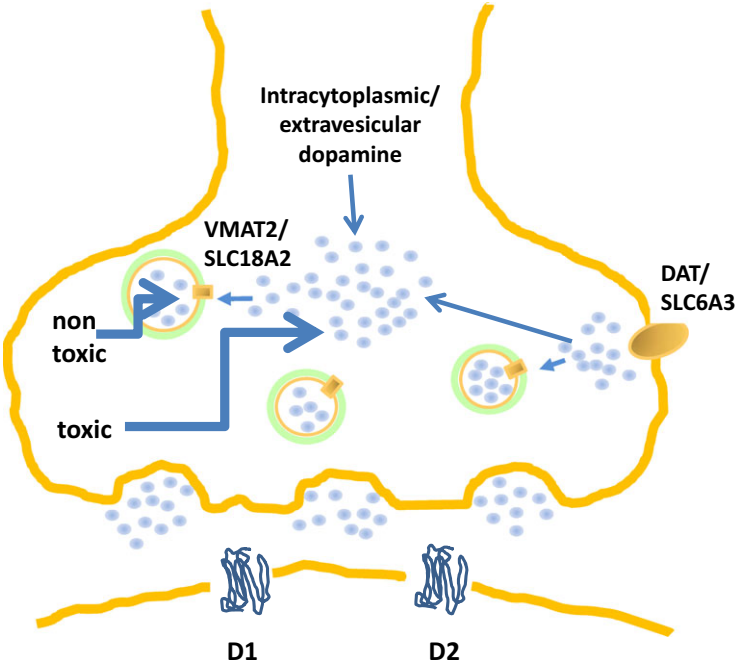

Figure 1. Dopamine terminal with plasma membrane SLC6A3/DAT and vesicular SLC18A2/VMAT2 transporters pumping dopamine into cytoplasmic compartments where it is toxic and vesicular compartments where it is nontoxic.

Mechanisms of Action of Toxicity from Dopamine, Amphetamine, MPTP, and 6-Hydroxydopamine

SLC6A3/DAT and SLC18A2/VMAT2 act to concentrate and compartmentalize dopamine, the MPTP active metabolite MPP^+, 6-hydroxydopamine, amphetamines, and other dopaminergic neurotoxins. These transporters regulate their substrates' concentrations in neuronal cytoplasm, where each can exert toxicities, and in synaptic vesicles, where most are less toxic (amphetamine's properties providing an informative exception; see below).[9] SLC6A3/DAT mediates neuronal uptake of these substances into the intracytoplasmic/extravesicular compartment with perhaps 0.5 μmol/L affinity for dopamine and 2.7 pmol/mg protein density in rodent striatum.[32–34] SLC18A2/VMAT2 pumps each of these substrates into synaptic vesicles with perhaps 1.5 μmol/L affinity for dopamine and 28.6 pmol/mg protein density.[35,36] SLC18A2/VMAT2 action can thus lower free cytoplasmic concentrations of these substrates and reduce experimental MPP^+ neurotoxicity. Amphetamine-like compounds classically cause dopamine release from synaptic vesicles, thereby elevating intracellular/extravesicular dopamine levels.[37] While there are other "nonclassic" ideas about amphetamine toxicities that include hyperthermia,[38,39] the major focus remains on dopamine redox contributions. Human amphetamine exposures are associated with significant changes in human dopaminergic markers[40] and in PD incidence/prevalence (see below and 41). Amphetamine-induced dopamine miscompartmentalization and subsequent toxicity display face validity as a model for dopaminergic aspects of PD pathogenesis that is at least as great as that displayed by other models for these selective dopaminergic aspects of PD neurodegeneration.[24]

Increased PD in Individuals Exposed to Amphetamine versus those Exposed to Cocaine or Controls

PD incidence/prevalence in control samples has been compared to rates in individuals with histories of cocaine use or of amphetamine use, though none of these reports details a precise amphetamine dose or a precise duration of exposure. PD patients attending San Francisco faculty practice clinics were eight times more likely to display histories of prolonged amphetamine use than their unaffected spouses.[42] California group practice attendees with amphetamine histories were more likely to have Parkinsonism diagnoses recorded subsequently than those identified by cocaine use (hazard ratio 2.44) or those identified based on a preceding appendectomy (hazard ratio 1.76).[43] Amphetamine-exposed individuals from a Utah registry displayed 2.8-fold increased risks for having PD diagnoses when compared to cocaine-exposed or to population-based samples.[44] Importantly, smoking (a PD protective factor[45]) was controlled for in this Curtin et al. work. Individuals prescribed amphetamine as therapy for narcolepsy subsequently developed Parkinsonism more frequently than expected by chance.[46] Amphetamine-exposed individuals displayed differences in substantia nigra volumes determined via ultrasound, finger tapping speed, and assessments of bradykinesia when compared with "other-drug" and "no-drug" control/comparison subjects[47] as well as the dopaminergic imaging changes noted above. In recently-reported work, stimulant-treated Utah registry individuals with adult diagnoses of attention-deficit hyperactivity disorder (ADHD) displayed up to eight fold increased risks of subsequent PD diagnoses.[48]

Taken together, this evidence supports the idea that increases in intracellular/extravesicular dopamine, mediated by amphetamine but not by cocaine, damage human dopamine neurons in ways that predispose to PD. Most of the amphetamine exposures identified in these reports were unlikely to have been lifelong. These results thus support the idea that even alterations in dopamine compartmentalization that occur during parts of the lifespan can exert lasting influences on these important human brain systems. The cocaine results from these epidemiological studies also provide important assurance about the relative lack of human toxicity from blocking the plasma membrane transporter SLC6A3/DAT alone. Indeed, trends identified in these data (e.g., hazard ratios 2.44 (cocaine) versus. 1.76 (appendectomy comparison group)[19,20]) provide some of the most tantalizing currently available human evidence that block plasma membrane dopamine transport might be neuroprotective, though appendectomy may provide a flawed control comparison group.

There are no clearcut epidemiological data that convincingly links chronic treatment with the plasma membrane transporter SLC6A3/DAT blockers methylphenidate or bupropion (see below) to altered PD risk though recent data that seeks to identify stimulant use may raise questions about methylphenidate use.[48] There are requirements for careful attention to covariates, including likely use of amphetamines in many who were prescribed methylphenidate for attention deficit hyperactivity disorder (ADHD) and cigarette smoking exposure in many who were prescribed bupropion to aid smoking cessation. Overall, existing epidemiological data does support dopamine compartmentalization hypotheses for regional dopamine pathology in PD.

Altered Effects of Dopaminergic Neurotoxins in Mice with Altered Levels of Expression of Dopaminergic Transporters

We and others have assessed the relationships between alterations in levels of expression of these transporters

and the extent of damage exerted by model dopaminergic toxins in vivo. Heterozygous SLC18A2/VMAT2 knockouts[29] with reduced compartmentalization into synaptic vesicles suffer more than twice as much dopamine cell killing by a modest MPTP regimen than wild-type littermates.[29] Enhanced methamphetamine toxicity in these mice is also consistent with easier amphetamine disruption of vesicular dopamine compartmentalization in mice with reduced vesicular transport capabilities.[28]

Mice with modestly increased SLC6A3/DAT expression in catecholamine neurons display more MPTP-induced dopamine cell losses than wild-type mice.[49] Mice that overexpress SLC6A3/DAT selectively in dopamine neurons display enhanced dopamine neuron losses with aging and elevated levels of markers for oxidative stress.[50] These and other data[51] increase confidence that differences in levels of expression of these transporters can impact dopaminergic cell toxicities of model neurotoxins and aging. They support the idea that increased in vivo and in vitro toxicity can result from increases in intracytoplasmic/extravesicular toxin (or dopamine) levels through augmented pumping into the cell by SLC6A3/DAT and/or reduced sequestration into vesicles by SLC18A2/VMAT2. While interpretation of these data may be limited by the validity of the model dopaminergic toxicities employed,[24] they are consistent with intracytoplasmic/extravesicular targets (e.g., electron transport chain/mitochondrial) for these toxins.

Altered Effects of Aging in Mice with Altered Levels of Expression of Dopaminergic Transporters

We have studied aged mice with heterozygous deletions of SLC18A2/VMAT2 or of SLC6A3/DAT, each reducing transporter expression to about 50% of levels found in wild-type mice.[51] Heterozygous SLC18A2/VMAT2 knockout mice display increased age-related loss of DA-mediated behaviors. By contrast, these features are preserved in aged heterozygous SLC6A3/DAT KO mice, when compared to modest to moderate losses in aging wild-type littermates. Neurochemical assessments confirm greater losses of ventral striatal dopaminergic markers in aged heterozygous SLC18A2/VMAT2 KO mice. These findings support the idea that lifelong alterations in levels of SLC6A3/DAT and SLC18A2/VMAT2 expression exert significant effects on age-related changes in dopaminergic function, likely due to their roles in regulating intracellular/extravesicular dopamine concentrations.

Transporter Expression by Dopaminergic Cell Groups and Cell Losses in PD Brains

Three dopaminergic neuronal groups display substantially different degrees of neuronal loss in PD brains studied postmortem. Quantitative studies document no detectable losses of arcuate neurons in PD brains.[52] 40–60% of ventral tegmental area dopamine neurons are lost from PD brains.[53] Most associated with much PD symptomatology are the losses of dopaminergic substantia nigra *pars compacta* neurons[54]; perhaps >80% of these are lost in brains of individuals who die with PD.[55] Explanations for PD pathogenesis must account for this selective pattern of dopamine neuronal losses.

These patterns of neuronal loss display striking parallels with their differential expression of the transporters that compartmentalize dopamine. In situ hybridization studies in rodents reveal significantly greater levels of SLC6A3/DAT mRNA expression in cells of the nigra compacta than in those of the ventral tegmental area, and very low levels of expression in arcuate hypothalamic neurons.[56] Sections from postmortem human brains also reveal more expression by nigra compacta than by ventral tegmental area neurons.[57,58] In remaining grossly intact PD nigral neurons, levels of expression of SLC6A3/DAT mRNA are lower than those of average nigral neurons sampled from matched control individuals. Levels of SLC18A2/VMAT2 expression have also been studied. SLC18A2/VMAT2 immunoreactivity is more abundant in human ventral tegmental area than nigra compacta neurons.[59] In situ hybridization studies also provide evidence for greater SLC18A2/VMAT2 mRNA expression in the ventral tegmental area than in nigra compacta cells.[56]

The distributions of Parkinsonian damage and transporter expression within the more dorsal versus ventral parts of the nigra compacta also provide support. Greater ventral tier losses in human PD have been correlated with greater expression of SLC6A3/DAT and lower expression of SLC18A2/VMAT2 immunoreactivity in nonhuman primate studies.[60]

These data support parallels between densities of SLC6A3/DAT expression and extent of dopaminergic cell loss in PD brains. Since much expressed SLC6A3/DAT and SCL18A2/VMAT2 protein is axonal, work on cell body levels of mRNA, and protein expression is limited by the assumption that these cell body levels predict axon/terminal levels. Parallels (nigral losses > VTA losses) in amphetamine-exposed rats do support the idea that disrupted compartmentalization can provide this PD-like pattern of cell type selectivity.[61] Of course, these findings do not account for losses of nondopaminergic neurons in PD brains.

F. Context This paper focuses on dopamine compartmentalization, the two transporters that are key to this process, evidence that features of this compartmentalization correlate with the dopaminergic cell type specificity of PD pathology and human and mouse support for the

idea that dopamine compartmentalization provides an approachable pharmacologic target for reducing cell type specific losses in PD. This pharmacological evidence is stronger than available physiological evidence. We lack details of the precise ways in which physiologically relevant concentrations of human intracellular DA exert toxicity in human aging and PD.

There are also potential interactions between dopamine compartmentalization and other pathophysiological mechanisms that are proposed for dopaminergic neuronal degeneration in PD. While detailed discussions are beyond the scope of this paper, there is no reason to believe that dopamine compartmentalization mechanisms could not interact with mechanisms involving synuclein,[62] autophagy[63] calcium,[64] axonal arbor size,[65] or mitochondrial oxidant stress.[66] There is no reason why potential dopaminergic neuronal protection from optimizing dopamine compartmentalization could not add to benefits from reducing dopamine redox cycling via monoamine oxidase inhibition or calcium compartmentalization strategies to reduce dopamine redox damage.

Dopamine compartmentalization ideas can be placed in the context of the genes that harbor common or rarer variants whose contributions to PD vulnerability have been identified by genome wide association (GWAS) or linkage/association in families.[67,68,69] A number of these genes, including ATP13A2, DDRGK1, GPNMB, GBA, GCH1, INPP5F, LRRK2, MAPT, PARK7, PINK1, RIT2, SNCA, STK39, UCHL1, and VPS35, are expressed either moderately or robustly in mouse ventral midbrain neurons in data available at [70]. However, there is no current strong documentation of nigra > ventral tegmental area > arcuate expression for any of these genes. Dopamine-selective mechanisms could be superimposed on the more generalized toxicities conferred by variation in these genes to provide the patterns of selective dopaminergic cell loss noted in PD brains.

Therapeutic Opportunities: Pharmacologies of Drugs that could Alter and Optimize Dopamine Compartmentalization

Reductions in dopamine concentrations in the intracellular/extravesicular compartment could come from drugs that (a) reduce SLC6A3/DAT-mediated dopamine uptake into this compartment (b) increase SLC18A2/VMAT2-mediated dopamine uptake from this compartment into synaptic vesicles. Added motivation for using such drug (s) could come from features that include well-understood use in humans, relatively long durations of action, modest toxicities, and modest, well-understood abuse liabilities.

We are fortunate that two drugs that block SLC6A3/DAT have long histories of use in humans. Each is documented to display modest toxicities and modest abuse liability. Both are available in extended release formulations. Each can act indirectly to stimulate actions of SLC18A2/VMAT2 in experimental animals. By contrast, while tetrabenazines block SLC18A2/VMAT2, we are not aware of any drug approved for human use that acts directly at SLC18A2/VMAT2 to enhance its activity.

Dopamine Transporter Blockers that Increase SLC18A2/VMAT2 Activity and have Substantial Human Use: Bupropion and Methylphenidate

Bupropion and methylphenidate each reduce dopamine uptake into intracytoplasmic/extravesicular compartments of dopaminergic neurons. They are each used, often chronically via extended release formulations, for on- and off-label clinical indications. In a recent year, more than 13 million methylphenidate and 24 million bupropion prescriptions were dispensed in the US.[71] Bupropion has successfully treated depression in Parkinson's patients[72,73]; methylphenidate has also been used in PD.[74] Human imaging studies have identified relationships between plasma levels of these drugs and SLC6A3/DAT occupancies.[75,76] There is thus deep human clinical experience to aid therapeutic application of insights into roles for SLC6A3/DAT and SLC18A2/VMAT2 in dopamine compartmentalization based on use of these drugs.

Methylphenidate potently blocks dopamine and norepinephrine transporters while inhibiting serotonin transport with lower potency.[77] Extended release methylphenidate preparations can provide 8–12 h efficacies.[78]

Bupropion blocks dopamine SLC6A3/DAT transport with more selectivity, though its major hydroxybupropion active metabolites display potency in inhibiting the norepinephrine SLC6A2 transporter as well.[79] The bupropion XL product label indicates 21 h half-life for the parent compound.

In experimental animals, both methylphenidate and bupropion can increase vesicular transport mediated by SLC18A2/VMAT2. Exposure to these drugs triggers cellular redistribution of SLC18A2/VMAT2 through changes in dopamine signaling that are attributed to actions at D2-like receptors.[80,81] If methylphenidate and bupropion increase SLC18A2/VMAT2 function in humans, there would be synergies with their abilities to inhibit SLC6A3/DAT. Both activities, taken together, would provide enhanced neuroprotection against dopaminergic toxicities mediated in intracytoplasmic/extravesicular compartments. Though many other compounds can inhibit SLC6A3/DAT, methylphenidate and bupropion gain salience for this work as the two SLC6A3/DAT inhibitors

that have been widely used in humans and may also increase SLC18A2/VMAT2 activity.

Neither bupropion nor methylphenidate causes high frequencies of any serious adverse side effect. Neither displays large abuse liability in many clinical settings.[82] Label information indicates that seizures are identified in about 1/1000 individuals who take sustained release bupropion. Users of either bupropion or methylphenidate can report nervousness, agitation, anxiety, insomnia, anorexia and/or weight loss, increased heart rate, and/or blood pressure. Despite the list of possible side effects, there is infrequent discontinuation based on side effects when either of these two drugs is used. Both of these drugs thus seem good candidates for repurposing to test their abilities to slow rates of selective loss of dopaminergic neurons in PD.

Concerns about Dopamine Miscompartmentalization Hypotheses for the Dopamine-Cell Type Specificity of Parkinson's Disease

Dopamine miscompartmentalization hypotheses for the dopamine-cell type specificity of Parkinson's disease have limitations. The results of work assembled in this review have accumulated over several decades; there is less current focus on dopamine systems in much thinking about PD pathogenesis than in the time during which this hypothesis was first proposed. A recent review of PD pathogenesis, for example, cites mitochondrial damage, energy failure, oxidative stress, excitotoxicity, protein misfolding/aggregation, impairment of protein clearance pathways, cell-autonomous mechanisms and prion-like protein infection without explicit reference to miscompartmentalization ideas.[83] Though there is no compelling evidence that L-dopa administration dramatically changes dopamine compartmentalization, lack of clear-cut evidence for L-dopa toxicity in clinical trials[84] has been interpreted by some to weigh against any pathophysiological role for dopamine toxicity. Availability of PD treatments that acceptably manage many problems caused by dopamine deficiency has supported ideas that drugs that aid dopamine neuronal survival would only provide modest changes in PD's natural history. The increasing focus on PD's multisystem nature[8] has been coupled with heightened realization that even striking success in aiding dopaminergic neuronal survival with methylphenidate, bupropion, or other agents that help to optimize compartmentalization would be unlikely to ameliorate all PD disability. Other therapeutic targets have been proposed and tested to varying extents. For example, there are both positive and cautionary notes concerning roles for calcium channel blockers in preventing degeneration of dopaminergic, and perhaps other, neurons.[85,86]

It is likely that agents that aim to ameliorate dopamine miscompartmentalization would have different impacts in different individuals. There are significant ranges of individual differences in expression of SLC6A3/DAT and SLC18A2/VMAT2 that are likely to have genetic and epigenetic bases.[28,87] Stem cells derived from discordant twins differ in expression of monoamine degrading enzymes.[88] Individual differences in bupropion metabolism are well documented to alter ratios of active metabolites that provide differential activities at different monoamine transporters.[89]

Drugs that reduce dopamine miscompartmentalization might have the best impact when applied early in the PD pathological process.[90] Unambiguous documentation of disease modifying effects of bupropion or methylphenidate will mandate experimental designs that parse out symptomatic effects.

Despite these limitations, however, there is increasing recent support for compartmentalization hypotheses from imaging studies,[40,91] hypothesis-testing epidemiology in amphetamine and cocaine users[43,44] and work with aging in mice that have constitutive differences in levels of transporter expression.[51]

Open Questions for Dopamine Compartmentalization Hypotheses for Dopamine Selective Lesions in PD

Questions that arise from the ideas above include (1) What chronic doses of SLC6A3/DAT blocking/SLC18A2/VMAT2 stimulating drugs can be tolerated by patients with early stage PD? When imaging detects asymptomatic dopaminergic lesions?; (2) Can bupropion and/or methylphenidate treatment beginning early in PD change compartmentalization sufficiently to slow losses of dopamine neurons and progression of dopamine-linked PD symptoms? Can treatment be more effective when initiated even earlier (e.g., when imaging of dopaminergic systems identifies preclinical dopamine depletion)?; (3) Do these compartmentalization hypotheses mandate increased surveillance of individuals with therapeutic and/or recreational exposures to drugs that miscompartmentalize dopamine (including amphetamine and the SLC18A2/VMAT2 blocker tetrabenazines)?; (4) How does dopamine compartmentalization-related toxicity differ in individuals with differing levels of transporter expression? With distinct PD-predisposing genetic variants? With distinct PD-related environmental exposures, including the robust protection from PD conferred by smoking and the more modest risks posed by pesticides, well water and rural environments in some epidemiological studies? With differing constellations of other PD medications?; (5) How could individuals' therapeutic responses be altered by

pharmacogenomic differences in genes whose products metabolize these drugs, including the cytochrome P450s that produce active bupropion metabolites? (6) Which of the postulated pathophysiologic mechanisms (e.g., redox, adduct formation, other) cause the toxicity from physiologically relevant concentrations of intracellular/extravesicular dopamine?

Compartmentalization of dopamine within neurons that utilize this important and toxic neurotransmitter now provides a well-supported hypothesis to help explain the selectivity of dopaminergic cell losses in PD brains. This hypothesis provides opportunities for novel therapeutic approaches to ameliorate the dopaminergic decline that occurs following PD diagnoses, based on repurposing available SLC6A3/DAT blocking/SCL18A2 enhancing drugs with which we have abundant human experience. Dopamine compartmentalization hypotheses also provide cautions about chronic use of drugs that physiologically or directly antagonize SLC18A2/VMAT2. Further animal model and human work in this area is thus highly justified. PD patients impatiently await safe and effective ways to slow the progression of the increasing, dopamine-linked disabilities that are among the unfortunate burdens imposed by this neurodegeneration.

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Author Contribution

GRU formulated the underlying hypothesis, wrote the manuscript and contributed to support of the hypothesis as noted in references.

Conflict of Interest

None.

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