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Heterogeneity of dopamine release sites in health and degeneration

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Abstract

Despite comprising only ~ 0.001% of all neurons in the human brain, ventral midbrain dopamine neurons exert a profound influence on human behavior and cognition. As a neuromodulator, dopamine selectively inhibits or enhances synaptic signaling to coordinate neural output for action, attention, and affect. Humans invariably lose brain dopamine during aging, and this can be exacerbated in disease states such as Parkinson's Disease. Further, it is well established in multiple disease states that cell loss is selective for a subset of highly sensitive neurons within the nigrostriatal dopamine tract. Regional differences in dopamine tone are regulated pre-synaptically, with subcircuits of projecting dopamine neurons exhibiting distinct molecular and physiological signatures. Specifically, proteins at dopamine release sites that synthesize and package cytosolic dopamine, modulate its release and reuptake, and alter neuronal excitability show regional differences that provide linkages to the observed sensitivity to neurodegeneration. The aim of this review is to outline the major components of dopamine homeostasis at neurotransmitter release sites and describe the regional differences most relevant to understanding why some, but not all, dopamine neurons exhibit heightened vulnerability to neurodegeneration.

Keywords

dopamine; neurotransmission; neurodegeneration; Parkinson's Disease; neuronal vulnerability; dopamine transporter

Ventral midbrain dopamine signaling

Dopamine in the brain

Earlier this year a novel theory of dopamine's action in the brain was put forth by Beeler and Kisbye [1]. Synthesizing elegant *in vivo* recording data that anatomically maps afferent input signals and dopamine neurons' firing response to stimuli, they argue that the cellular and subcellular specializations of dopamine neurons allow them to act as high volume sampling

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programs [2-4]. Thusly, coordinated increases in randomly sampled afferent activity signal consensus to dopaminergic subsets; consensus signals when to increase brain dopamine tone to regionally inhibit or disinhibit synaptic signaling and plasticity [1-4].

Dopamine signaling from the ventral midbrain can be grossly simplified into two components: 1) acting as a neurobiological substrate conveying the timing of salient stimuli, and 2) encoding patterns for selection and execution of goal-oriented movement [1,5,6]. This functional bifurcation maps onto the predominant anatomical distinction between midbrain dopamine neurons, the ventral tegmental area (VTA, salience) and substantia nigra pars compacta (SNc, movement)[7,8]. VTA dopamine neurons comprise the mesocortical and mesolimbic dopamine circuits; these cells increase firing activity to raise dopamine levels in brain areas important for threat or reward recognition and the subsequent learning that would encode an aversive or seeking habit [9]. SNc, or nigrostriatal circuit, dopamine neurons project through the direct and indirect pathways of the basal ganglia, with the ultimate output being neurons in the motor cortex to control goal-oriented movement [6]. It is a subset of these nigrostriatal neurons that have been consistently shown to be more sensitive to aging and neurodegeneration [10-12]. Interestingly, these two systems can redistribute encoding between their respective striatal targets: when a learned behavior becomes habitual, the encoding of the task is shunted away from dorsomedial striatum (associative) to the dorsolateral striatum (sensorimotor)[13]. Also of note, this neural merging point creates a dovetail in diseases related to dopamine neuron dysfunction. For example: in Parkinson's Disease (PD), a movement disorder induced by degeneration of SNc dopamine neurons, increased prevalence of impulse control disorders has been observed [14]. Other non-motor symptoms of PD including depression and working-memory are currently under investigation as measurable markers of disease progression and subtype [15,16]. However, the linkages between physiological diversity to shared and distinct facets of dopamine-related disorders are not completely characterized.

In contrast from point-to-point neurotransmission, dopamine signals en masse. Synchronized subcircuits of dopamine neurons establish extracellular concentrations throughout their respective projection area. At terminal fields in the dorsal and ventral striatum, medium spiny neurons (MSNs) express D1-like ($G\alpha_{s/olf}$ coupled) and/or D2-like ($G\alpha_{i/o}$ coupled) G-protein coupled receptors[17-19]. In nigrostriatal circuits, these MSN populations represent the flow of information through the direct pathway (action initiation, D1) and the indirect pathway (action inhibition, D2) respectively [7,20]. Within and beyond the striatum, activated D1-like and D2-like receptors modulate adenylyl cyclase activity to facilitate or dampen synaptic signaling respectively [21]. Dopamine neurons also express D2 autoreceptors which act as negative feedback agents on cell firing and dopamine release [22,23]. Once activated, D2 autoreceptors reduce dopamine release and promote reuptake by inducing membrane hyperpolarization and reducing Ca^{2+} influx [24,25]. However, the relative contribution of D2 autoreceptor activity to extracellular dopamine levels varies considerably between species and brain regions [24,26-28].

Aberrant dopamine signaling has been linked to many psychiatric and neurological disease states including schizophrenia, bipolar disorder, major depressive disorder (MDD), attention deficit and hyperactivity disorder (ADHD), PD, Huntington's disease (HD), and HIV-1

associated neurodegeneration (HAND) [29-34]. The scope of the current review is to outline release-site vulnerability most relevant to the loss of dopamine neurons in idiopathic PD. Understanding PD-related factors may also provide insight as to why healthy humans appear to display an invariable gradual loss of dopamine tone through aging, albeit with a pattern that is distinct from neuronal loss in PD [35]. Additionally, ~15% of healthy individuals examined postmortem exhibit post-mortem PD brain pathology - namely proteinaceous alpha-synuclein aggregates termed Lewy bodies (LBs) - that would suggest the presence of a dopaminergic disorder with no such clinical manifestation [36-39]. Despite this, presence of LBs in dopaminergic neurons remains a gold-standard confirmatory symptom in PD, although whether LBs represent cytotoxic aggregates or neuroprotective sequestration bodies remains an active debate [40-42]. These data obfuscate the roles of proteostatic stress and LB pathology in dopaminergic dysfunction and degeneration, despite numerous linkages between LB-like alpha-synuclein aggregates and neuronal dysfunction and loss in model systems [43-47]. Nevertheless, SNc dopamine neurons generally exhibit greater levels of neurodegeneration in human disease and animal models of dopaminergic neurodegeneration [11,48,49]. Thusly, it stands to reason that some facets of SNc dopaminergic physiology in and of themselves lead to heightened vulnerability and cell death during aging [12].

Feed-forward dopamine signaling via intrinsic pacemaking

Dopamine neurons are intrinsic pacemakers, with their slow (2-4 Hz) rhythmic activity first described in the rat SNc by Grace and Bunney in 1984 [50]. It is hypothesized that this feedforward control of activity has been conserved because a tonic concentration of dopamine – irrespective of external stimuli – is advantageous should a rapid movement such as predator evasion become necessary with little warning [12]. Additionally, dopamine neurons can fire in rapid bursts in response to relevant salient stimuli [51]. This transient increase in firing induces a temporally-precise rise in dopamine concentrations allowing a specific stimulus/response to be encoded and, if applicable, entrained. As described earlier, anatomically discrete dopaminergic subcircuits exhibit coordinated rises in firing rates to raise dopamine concentrations in accordance with the timing of afferent input agreement [1]. This can be easily observed in the context of reward prediction error, during which rewards of greater uncertainty elicit a larger increase in VTA cell firing – putatively to strengthen synapses important for the reinforcement of an unexpectedly (i.e. novel) favorable behavior [5,52].

SNc and VTA dopamine neurons exhibit fundamental differences with regards to their ionic control of pacemaking activity [53]. In the VTA, slow rhythmic firing is predominantly driven by TTX-insensitive Na^+ channels and regulated by a dynamic A-type K^+ current [54,55]. In the SNc however, voltage-gated Ca^{2+} channels carry the majority of current that drives pacemaking and frequency is similarly regulated by A-type K+ currents [56-58]. Also of note, SNc and VTA dopamine neurons exhibit different sag-currents in response to hyperpolarization, a key pacemaker current often used to identify dopaminergic neurons as such [59,60]. Studies in mice have shown that SNc and VTA dopamine neurons exhibit inter- and intra-regional differences in tonic firing rates, with lateral SNc neurons showing the highest firing rate, and medial VTA neurons showing the lowest [59]. Consistent with their basal differences, SNc and VTA dopamine neurons exhibit distinct responses to electrical

and pharmacological stimulation *ex vivo,* but this has been shown to vary across common murine models (namely rats, mice, and guinea pigs) [27,28,61].

Pacemaker activity has been heavily implicated in SNc dopamine neuron's heightened vulnerability profile [62]. Specifically, it has been shown in mice that pacemaking-induced Ca^{2+} influx leads to the generation of reactive oxygen species in the mitochondria of SNc dopamine neurons [62-65]. This reactive oxygen species generation likely underlies the high level of mitochondrial DNA mutations observed in human SNc neurons, which would in turn gradually compromise the energetic competence of the cell [66,67]. VTA neurons are seemingly spared from this energetic compromise not only because they are less reliant on Ca^{2+} channels for pacemaking, but also because they are enriched with the calcium binding protein calbindin [54,8,68]. Degeneration-sensitive SNc neurons, in contrast, map to one end of a calbindin expression gradient at which the protein is completely absent [48,69]. Importantly, the inverse relationship between calbindin expression and degeneration-sensitivity of dopamine neurons has been observed in humans as well as rodents and non-human primates [69-71,68]. In the SNc of mice, backpropagating action potentials were shown to induce supralinear increases in dendritic Ca^{2+}, indirectly suggesting a heightened sensitivity in these cells to deviations from homeostatic setpoints [72]. It should be noted however that the impact of pacemaking and Ca^{2+}-linked energetic decline is only one component of a multi-faceted intrinsic vulnerability profile, which also includes the large projection area and intense axonal arborization of SNc neurons, expression of the aggregation-prone protein α-synuclein, and the oxidant qualities of cytosolic dopamine [12,64,65,73,74].

Synaptic specializations & dysfunction in dopamine neurotransmission

Dopaminergic neurons are specialized to receive high volumes of afferent information and synthesize a responsive and advantageous neuromodulatory tone through a large projection area [1,2,73]. Important to this is a relative sparsity of bona fide dopamine synapses with traditional presynaptic proteins and clear post-synaptic targets [75-78]. Instead, release sites can be seen in the general vicinity of post-synaptic medium spiny neurons that are putatively being influenced by brain region-specific changes in dopamine tone [75,79]. There is considerable redundancy in this system: in rats, one SNc dopamine neuron is estimated to influence ~75,000 striatal MSNs [73]. Subsequent to this, any given individual striatal MSN is influenced by dopamine released from 95-194 dopamine neurons [73]. The lack of canonical synaptic release sites is coupled to a low probability of release for dopamine containing vesicles, allowing an accurate scaling of neurotransmitter released as a function of changing firing frequencies [77,80,81]. Taken together, these data can explain the long-standing observation that dopamine neurons harbor a "reserve pool" of dopamine vesicles that are highly insensitive to stimulation, as well as the more recent finding that up to 60% of dopaminergic synaptic release sites are functionally silent when stimulated [82,83,81,78].

Neuronal dysfunction at axonal and terminal release sites is believed to precede somatic dysfunction and degeneration in SNc dopamine neurons [84-87]. As mentioned above, large and highly arborized axons constitute one facet of SNc dopamine neurons' intrinsic-vulnerability profile, putatively through an increase in surface area for active release and reuptake processes with little concomitant increase in cell volume to hold adequate

bioenergetic machinery [65,73]. These axons are also unmyelinated, further increasing the energetic demand on the cell [88-90]. Supporting this hypothesis, experimental depletion of axonal mitochondria by inhibiting mitochondrial fission induces a retrograde degeneration of SNc dopamine neurons [91]. These data are consistent with proposed mechanisms of *pink1*- and *parkin*-linked familial PD, as both genes encode for proteins responsible for regulating mitochondrial fission[92-94].

Studies conducted *in vitro* examining the staging of α-synuclein aggregation indirectly support the argument for retrograde dysfunction: synaptic and axonal accumulation of insoluble α-synuclein inclusions precede that seen in the soma of cultured mouse hippocampal neurons [87,95,96]. The physiological role of α-synuclein in synaptic neurotransmission is not fully characterized, although it has been shown that this small (14 kDa) intrinsically-disordered protein senses and binds curved membranes like those of synaptic vesicles [97-99]. α-synuclein is hypothesized to directly affect vesicular dynamics by organizing exocytotic machinery as well as through direct interactions with vesicles themselves to expand the size of the vesicular fusion pore thereby promoting exocytosis [100-103]. Importantly, synaptic and axonal α-synuclein accumulation lowers the expression of pre-synaptic proteins required for canonical neurotransmission, decreases the frequency of mEPSCs in post-synaptic target neurons, and inhibits vesicle recycling dynamics [87,96,104]. The observed effect of α-synuclein aggregation on pre-synaptic function could carry profound ramifications in any neuronal environment, but the oxidant properties of dopamine may further amplify downstream cellular insults in SNc dopamine neurons [105-107]. Given the heterogeneity in vesicular dynamics at dopamine release sites, it is possible that either silent release sites harboring stagnant vesicles, or active release sites undergoing near-constitutive release and recycling, may create an environment that facilitates α-synuclein aggregation capable of compromising synaptic function.

The sum total of these factors suggests proper handling of dopamine by SNc neurons at release sites as a critical homeostatic setpoint, deviation from which can disproportionately compromise long-term cell viability. Herein, we aim to outline the major proteins responsible for dopamine homeostasis at release sites in dopamine neurons, with specific attention paid to how they differ between the disease sensitive SNc and relatively robust VTA dopamine neurons. We discuss the basic physiological role, regional differences, and contributions to disease of four key proteins involved in synaptic dopamine synthesis, storage, and recycling. Using that information, we also present a cell-level view of how each individual component's behavior may create a vulnerable SNc dopamine phenotype. Finally, we discuss the benefits and pitfalls of the current state of modeling synaptic physiology in dopamine neurons, and how the use of new temporally and spatially resolved technologies can be applied to remaining knowledge gaps.

Synaptic regulation of dopamine homeodynamics

Signal termination and recycling of dopamine by the dopamine transporter

The dopamine transporter (DAT, *SLC6A3*) is a sodium/chloride-dependent neurotransmitter transporter that is responsible for terminating dopaminergic signals by reuptaking dopamine molecules released into the extracellular space[108]. This process has been argued to be the

foremost modulator of brain dopamine tone [26,28,109]. DAT also exhibits efflux, a process in which the direction of dopamine transport is reversed and transporters begin releasing dopamine into the extracellular space [110]. Efflux of DAT is the underlying mechanism of the widely prescribed and abused psychostimulants known as amphetamines [110-112]. DAT is expressed at the cell bodies and along the dendritic field of dopamine neurons, which also serve as locations of dopamine release and signaling [113,114]. However, the precise contributions of DAT-mediated dopamine efflux and reuptake to somatodendritic release are not fully characterized [115-117].

Multiple mutations in the gene encoding DAT (*SLC6A3*) have been identified in humans. The phenotypic spectrum varies widely and includes: infantile parkinsonism and dystonia, Attention Deficit and Hyperactivity Disorder (ADHD), Schizophrenia, and Autism Spectrum Disorder [32,118-122]. Individuals carrying homozygous loss-of-function missense mutations at the DAT allele develop parkinsonian and dystonic motor deficits (known as dopamine transporter deficiency syndrome) as early as one year in age [118,120,123]. Currently, symptomatic relief is by-and-large unattainable in these patients due either to a lack of clinical efficacy or the presence of adverse side effects [120]. In two individuals, compound heterozygous mutations in the gene encoding DAT gave rise to motor symptoms at three and four years of age[123]. Interestingly, mice that do not express DAT show a transient hyperactive phenotype, but ~one-third (36%) of these mice go on to develop severe and progressive motor deficits that result in mortality by one year of age [124,125]. Thusly, evidence from both animal models and human mutations establish a critical role of dopamine recycling in maintaining long-term brain dopamine levels.

It is important to note that decreased DAT expression or DAT knockout yields protection against experimental dopaminergic neurotoxins such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyradine (MPTP) and 6-hydroxy-dopamine (6-OHDA), but these molecules are transported into cells by DAT itself [126]. Therefore, DAT-deficient animal models of PD are unlikely to relate to the innate pathogenic course of PD in humans [127]. However, DAT activity in the SNc vs VTA has been examined to determine if differential transport activity may contribute to the selective sensitivity of SNc cells. Indeed, data from SNc- or VTA-enriched neuronal cultures have shown that SNc neurons exhibit higher uptake of a DAT-specific fluorescent molecule [64]. This correlates with what is known about *in vivo* DAT behavior in rats, in which the clearance rate of dopamine is greater in SNc efferent regions (i.e. striatum) than in VTA efferent regions (i.e. nucleus accumbens) [109,128,129]. These data imply that the potential role of neurotoxin accumulation through DAT would be expected to impact SNc dopamine neurons more than their VTA counterparts. And while it is notable that neurotoxin induced parkinsonism has been documented in humans, and occupational exposure to neurotoxic pesticides increases the risk of developing PD, the degree to which toxin uptake contributes to the death of SNc cells on a large scale with no conspicuous exposure may be difficult to determine [130-132].

Production – the role of tyrosine hydroxylase in dopamine homeostasis

Tyrosine hydroxylase (TH) is the rate limiting enzyme of the dopamine synthesis pathway, but has also long served scientific ends as the gold standard for immunolabeling of

dopamine neurons [133-135]. In humans, TH is present in cell bodies, dendrites, axonal projections, and terminal release sites of both SNc and VTA dopamine neurons [136-138]. This suggests that dopamine is not synthesized in any one compartment of dopamine neurons but can be made locally for release throughout the cell. However, evidence from model systems reveals subcellular specialization does occur, with TH enrichment shifting from cell bodies to terminals during the early stages of growth (~DIV 3) in organotypic rat cultures [139]. This correlates with what is known about dopamine concentrations in fully developed *ex vivo* mouse brain slices, wherein the measured dopamine content is ~20 fold higher in the striatum relative to the SNc and VTA [140]. The relative enrichment of TH in terminals vs. soma remained detectable in 12 month old rats, with the striatum exhibiting the highest amount of TH protein measured [141]. This sheds light on a potential impact of *de novo* dopamine production on cellular vulnerability, as the most TH-enriched brain region maps onto the most sensitive to degeneration in PD. Relative TH abundance shows an opposite pattern in the soma though: tissue obtained from the VTA contained ~3 fold higher levels of TH than tissue obtained from SNc counterpart regions [141]. The nucleus accumbens (NAc), a major projection region of the VTA immediately ventral to the striatum, showed similarly low levels of TH to that measured in the soma of SNc neurons [141]. Taken together, these data tell us that the developmental shift of TH enrichment from somatic to terminal regions occurs strongly in SNc dopamine neurons (~7 fold higher TH in striatum than SNc), but VTA counterparts maintain an opposite, but more modest, enrichment of TH at the soma (~2.5 fold higher TH in VTA than NAc) [141]. However, physiological and pathological ramifications of this paradoxical TH distribution remain incompletely understood. Taken in conjunction with the fact that SNc neurons exhibit higher DAT uptake activity, it is potentially the case that these cells operate at a higher dopamine “steady-state” concentration than VTA dopamine neurons. This idea is supported by intracellular patch electrochemistry measurements done in cultured dopamine neurons, in which SNc neurons harbor two to three fold higher levels of cytosolic dopamine than VTA neurons [48]. Whether or not these somatic measurements would remain consistent with free dopamine levels in terminal fields is not yet known. Regardless, these data suggest that SNc dopamine neurons may not only be subject to relatively higher levels of oxidative damage due directly from dopamine’s oxidant potential, but may also be more sensitive to any insult that would disrupt or diminish dopamine levels in these cells.

Tyrosine hydroxylase deficiency (THD), a rare autosomal recessive disorder caused by loss-of-function mutations in the gene encoding *TH,* most commonly presents as an early onset motor disease responsive to exogenous L-DOPA treatment [142]. Severity of symptoms follows a gene dose-dependent pattern, with homozygous mutation carriers presenting with sever deficits during infancy, while heterozygotes will typically not show symptoms until adolescence [143]. THD is almost exclusively caused by missense mutations in the *TH* gene or promoter, suggesting that mutations that induce a more drastic phenotype are heavily selected against and will result in lethality before a disorder can be identified [143]. Lending credence to this hypothesis is data from transgenic mouse models, in which full knockout of the *TH* gene resulted in severe embryonic lethality [144,145]. Further, transgenic mice expressing hypofunctional TH exhibited gross motor defects despite anatomically normal dopaminergic innervation [146].

THD is a rare disorder, however, a loss of TH immunoreactivity without a concomitant loss of dopamine neurons is a common observation across multiple disease states. For example, post-mortem samples from individuals with PD show that melanized SNc dopamine neurons lose their TH immunoreactivity before the cell degenerates *per se* [137]. A similar pattern was observed in mice overexpressing the HIV-1 associated protein Tat: Tat overexpression induced a loss of SNc TH immunoreactivity yet a general neuronal marker (NeuN) revealed that there was no gross loss of neurons in this region [34]. Mechanistically, the latter case can be explained by a direct inhibition of *TH* gene expression by HIV-1 Tat [147]. In PD however, the cascade of events that precede and induce a loss of TH immunoreactivity is poorly understood. Similarly, it remains unclear what the functional properties of these TH-negative melanized dopamine neurons are or if they can be rescued to restore their TH expressing phenotype.

Packaging cytosolic dopamine via vesicular monoamine transporter 2

Vesicular monoamine transporter 2 (VMAT2, *SLC18A2*) acts to sequester cytosolic dopamine into vesicles for exocytotic release into the extracellular space[148]. In both the NAc and dorsal striatum, VMAT2 is localized to small synaptic vesicles (SSVs) where it packages dopamine for canonical vesicular exocytosis [149]. In dopaminergic cell bodies, VMAT2 localization is seen on lateral saccules of the golgi apparatus and tubulovesicular organelles resembling smooth endoplasmic reticulum (sER)[149]. In mesolimbic dopamine neurons (ie. NAc terminals / VTA cell bodies) VMAT2 is also observed on dense core vesicles, though these vesicles are absent in the SNc [150]. Similar to the above described TH expression data, quantitative analysis suggests that VTA cell bodies harbor significantly higher amounts of VMAT2 than their SNc counterparts[149]. As described earlier, it is theorized that one factor contributing to the heightened vulnerability of SNc dopamine neurons is a high cytosolic dopamine concentration [48]. A relative scarcity of SNc VMAT2 expression at cell bodies may provide a mechanism underlying this phenomenon, however it is also possible that the concomitant lower TH expression in the SNc dopamine neurons compensates for lower VMAT2 levels.

Similar to TH, mutations in the gene encoding VMAT2 are rare and individuals with these mutations present with a severe early-onset parkinsonism [151]. These symptoms were coupled to global developmental deficits, sleep disruption, and reduced urinary dopamine [151]. In contrast to *TH* mutations, treatment with exogenous L-DOPA induced a worsening of symptoms which returned to baseline levels when treatment was halted [151]. Functionally, this mutation was shown to result in a hypofunctional transporter, and treatment with a dopamine receptor agonist provided substantial and sustained improvements in symptoms [151]. In addition to bona fide mutations, rare single nucleotide polymorphisms (SNPs) have been identified in the *SLC18A2* gene in individuals with PD and micropthalmia [152,153].

VMAT2 knockout mice survive embryonic development and have morphologically normal DA neurons, but move and feed little and die shortly after birth [154,155]. In contrast, animals heterozygous for the knockout express ~50% of the VMAT2 seen in wild-type counterparts and exhibit a normal lifespan [154,156]. VMAT2 heterozygous knockout animals display comparatively subtle dopaminergic defects however, including reduced reward conditioning

in response to amphetamine and increased vulnerability to the dopaminergic neurotoxin MPTP [156]. To bridge the gap between the poorly viable full knockouts, and the largely unperturbed heterozygotes, a VMAT2 deficient mouse line expressing ~5% of wild-type levels was later generated and characterized [157]. These VMAT2 deficient mice were grossly normal and lived into adulthood, but exhibited age-dependent motor deficits and dopaminergic degeneration (similar to that seen in PD) [157]. Unlike the cases observed in humans with mutations in VMAT2, treatment with exogenous L-DOPA led to a reversal of the motor deficits observed in aged VMAT2 deficient mice [157]. Also of note here, is the coexpression of VGLUT2 in midbrain dopamine neurons, a vesicular transmitter for the neurotransmitter glutamate. It has been observed that neurotoxic insult increases the proportion of SNc dopamine neurons that coexpress VGLUT2, and overexpression of VGLUT2 induces dopaminergic neurodegeneration and concomitant motor deficits [158]. However, the mechanisms of VGLUT2-induced neurotoxicity, and how an interplay with VMAT2 contributes to this, are not fully characterized.

Regulation of neuronal firing and extracellular DA by D2 autoreceptors

Dopaminergic neurons express D2 receptors (D2Rs) that act as a negative feedback conduit to reduce neuronal firing when activated by extracellular dopamine [159,25,22]. Two isoforms of D2Rs generated through alternative splicing – $D2_{Long}$ and $D2_{Short}$ – are expressed in the mammalian CNS[160]. Data from $D2_{Long}$ knockout and total D2R knockout mice suggest that presynaptic autoregulation of dopamine neurons is predominantly carried out by the $D2_{Short}$ isoform[161,162]. These $D2_{Short}$ autoreceptors are coupled to G-protein activated inwardly-rectifier K^+ (GIRK) channels which hyperpolarize the cell and inhibit firing [25,163,164]. Membrane hyperpolarization has also been shown to increase surface DAT, suggesting a dynamic feedback system specialized for large changes extracellular dopamine [165]. This is directly supported by *in vivo* data showing that while dopamine reuptake is accelerated under continuous stimulation to meet demand, there is no such acceleration in mice lacking D2Rs [166]. Additional molecular and biophysical underpinnings of D2Rs positive modulation of DAT-mediated uptake have also been identified in heterologous expression systems [167-169]. D2R activation has also been shown to reduce Ca^{2+} influx through voltage-dependent Ca^{2+} channels in dopamine neurons, thereby limiting vesicular neurotransmitter release [170]. The combination of firing inhibition, uptake enhancement, and reduction of Ca^{2+} influx constitutes a multi-pronged response through which D2 autoreceptor activation can reduce extracellular dopamine levels [24,170]. Notably, the D2 antagonist sulpiride is commonly used as an antipsychotic in the treatment of schizophrenia, although its clinical efficacy has been debated [171].

D2R activation threshold and relative contribution to extracellular dopamine levels vary considerably between brain regions and model species [26,28,172,173]. Studies have shown distinct D2R-mediated autoregulation when comparing SNc and VTA dopamine neurons [27]. Results gathered using *ex vivo* voltammetry reveal that while terminal regions of SNc and VTA dopamine neurons are strongly regulated by D2R activation, dopamine release at the cell bodies is only altered by D2R activity in the SNc [27,174]. This was further dissected using single-cell electrophysiology, which identified mesocortical (VTA to prefrontal cortex) dopamine neurons as the only subtype that did not exhibit a GIRK2-mediated

hyperpolarizing current in response to D2R activation [173]. These mesocortical neurons appear to map to a location of the VTA that was identified as harboring TH(+)/D2(−) dopamine neurons, potentially explaining their lack of autoinhibition[175]. A second potential contributor to this is the relatively higher expression of GIRK2 in SNc cell bodies than in the VTA [8,173,176]. Similar to calbindin, this is consistent with a developmentally-determined gradient of GIRK2 expression, although in this case enriched GIRK2 expression maps to disease-sensitive SNc dopamine neurons[177]. The VTA also differs in its expression of the GIRK3 subunit, which can form heteromeric channels with GIRK2 that are less efficacious at coupling to D2Rs [178-180]. One last potential difference between the SNc and VTA is D2-mediated downregulation of TH [181,182]. Due to the contrasting subcellular distribution of TH and graded expression of D2R in the SNc vs. VTA, it is likely that D2-TH interactions asymmetrically shape dopamine synthesis and release in the two regions [141,182]. Owing in part to the existence FDA-approved therapeutics that can safely and specifically target D2Rs, these receptors may prove a potent therapeutic target to exogenously modulate dopamine homeostasis at release-sites and cell-wide.

Tying together synaptic phenotype of midbrain dopamine neurons

Ontology begets physiology – graded heterogeneity in midbrain dopamine neurons

As alluded to earlier, multiple disease relevant proteins (ie. calbindin, GIRK2) exhibit an expression gradient across midbrain dopamine neuron populations [8,68,136]. This strongly suggests regions are specified during early development, owing to the role of signaling centers and diffusion gradients in brain organization [183,184]. Indeed, the developmental fate of dopamine neurons appears to be established prior to birth and heavily regulated by numerous transcription factors (TFs) after birth [184,185]. Non-TF proteins that exhibit an expression gradient can readily be correlated with the relative disease sensitivity of dopamine neurons, albeit with different degrees of causality suggested [71,136,186]. However, a direct role of TF-mediated maintenance of dopamine neurons has also been proposed and mutations in these genes have been linked to PD [187]. Irrespective of any individual component's causality, understanding the dopaminergic nuclei of the midbrain to be organized in a graded fashion may provide important clues as to how a subset of SNc neurons have evolved to be so susceptible to neurodegeneration.

The nigrostriatal subpopulations of dopamine neurons that appear most sensitive to degeneration in human diseases states exist at an anatomical boundary – the ventrolateral-most portion of the SNc[188]. This pattern of loss in the SNc has also been mapped onto animal models of dopaminergic degeneration, with the dorsolateral region of the SNc proving most vulnerable [69]. These neurons are likely to comprise one end of developmental expression gradients, which is partly supported by rodent immunolabeling data of calbindin expression (absent in these neurons), and GIRK2 (enriched in these neurons) [8]. And much like the expression of specific proteins, physiological processes can be observed as changing through dopamine neurons in a graded fashion. For example, the tonic firing rate of dopamine neurons is highest in the most lateral subset (lateral SNc), and lowest in the most medial subset (medial VTA) [59]. This is coupled to an increased membrane resistance, lowered membrane capacitance, and smaller cell size in the medial VTA compared with it's

lateral SNc counterparts [59]. Sensitivity to degeneration also presents as a gradient throughout midbrain dopamine neurons [69,177]. However, the dichotomous readout of living vs. dead cells, or TH immunoreactive vs. non-immunoreactive, may preclude the appreciation of graded changes in dopamine neuron health and pathophysiology. Thusly, processes that can be studied in living dopamine neurons along a continuous spectrum - including but not limited to synaptic dopamine handling and vesicular dynamics – may allow for a better understanding of which cell populations undergo changes in age-related dopaminergic neurodegeneration like PD.

Potential contributions of synaptic phenotypes to selective dopaminergic degeneration

Adding in the above described synaptic phenotype data, SNc terminal fields harbor the highest concentration of TH observed across all regions studied, but relatively low levels of somatic TH expression [141]. Although this data does not specify a location of terminal fields that could provide information about SNc subpopulations, it is not unreasonable to think that similar to other graded identifiers in dopamine neurons, the SNc neurons most susceptible to degeneration exist at one extreme. This leads to the hypothesis that the most sensitive SNc neurons will express high levels of TH in terminals and suggests a heightened reliance on *de novo* dopamine synthesis to carry out signaling. It should follow then, that these neurons will harbor similarly high amounts of VMAT2 in terminals, to manage *de novo* dopamine packaging, and lower DAT expression or activity due to less reliance on transmitter recycling. However, the opposite is true for DAT expression – SNc neurons show greater DAT-mediated substrate uptake when measured at the cell body, and their projection regions show greater rates of dopamine clearance following release [64,109,128,129].

This now establishes SNc dopamine neurons as a population that signals using relatively more dopamine resources than their VTA counterparts (increased production signified by TH expression, increased reuptake and recycling signified by DAT behavior). Therefore, these neurons can be phenotyped as “dopamine-high” (DA_{high}) cells, characterized by relatively heightened levels of steady-state dopamine production, packaging, and recycling. This phenotype is also indirectly supported by single-cell cytosolic dopamine measurements in SNc and VTA specific mouse cultures [48]. A DA_{high} phenotype may underlie these neurons’ higher spontaneous firing frequency, potentially a mechanism to purge adequate amounts of *de novo* synthesized dopamine and prevent cytosolic accumulation. Given the high tonic firing rate and TH enrichment in terminals, it is also plausible that the MSNs receiving input from DA_{high} SNc neurons are adapted to relatively high tonic concentrations of dopamine, which as described above, may render them disproportionately sensitive to any insults that disrupt dopamine handling. If true however, therapeutic restoration of dopamine signals would require careful determination of the appropriate homeostatic setpoint – either a minimal concentration that can promote appropriate brain signaling while preserving cell health, or a higher concentration that aims to re-establish physiological levels. This may be difficult to determine owing the earlier described redundancy in dopaminergic connections, however understanding how different dopamine setpoints alter neuronal physiology and response to insult through time may prove critical to finding an effective degeneration modifying agent.

Modeling the dopamine handling phenotypes of the SNc and VTA

As outlined, synaptic dopamine handling can be linked to dopaminergic sensitivity to degeneration as well as other dopamine related disorders [48,64,121]. This is also an appealing avenue of investigation due to a growing toolbox of optical sensors and fluorescent dyes generated to examine dopamine neurotransmission. These include probes to measure vesicular release and recycling, DAT trafficking and uptake, and dopamine receptor activation (D1R and D2R) [81,77,189-192]. Depending on the model system, these tools provide minimally-invasive measures of synaptic dopamine dynamics. This can be conducted using a large field of view to compare anatomically distributed dopamine neurons, or with resolution that reaches single release sites in more defined anatomical areas [190,81].

A remaining challenge in studying age-dependent dopamine loss is the limited availability of animal models that adequately capture the complexity of processes that take place over decades in the human brain [12]. Neurotoxins selective for dopamine neurons – most commonly MPTP and 6-OHDA - have been exploited in mice, rats, and non-human primates to investigate pathophysiological processes involved in dopaminergic neurodegeneration [193-195]. And while meaningful refinements have been made, these neurotoxins are generally thought to induce a more acute cascade of pathophysiology preceding cell death than that which may take place over a full lifespan in the human brain [196]. Also as described, both of these toxins have been shown to be transported into dopamine neurons via uptake by DAT, preventing examination of endogenous components of DAT activity to neurodegeneration [127,197]. Thusly, it remains difficult to know how closely the cell death seen in these models matches that which takes place in human neurodegeneration, and how a therapeutic window can be scaled from these models to human disease. However, dietary and peripheral neurotoxin models that more closely replicate patterns of pathology progression seen in human disease represent a promising and growing field to investigate dopamine neurodegeneration [198,199].

In addition to dietary and peripheral insult models, transgenic mouse models have become an important tool for investigating gradual and SNc-specific dopamine neuron cell-death [200-203]. A subset of these models are based around genetic mutations that have been linked to PD dopamine neurodegeneration in humans [43,204,205]. Transgenic models with disrupted mitochondrial physiology – most prominently the MitoPark mouse strain – have been generated to mimic the energetic burdens hypothesized to impinge on SNc dopamine neurons [206-209]. Briefly, deletion of the mitochondrial transcription factor A from dopamine neurons results in gradual and SNc-selective dopamine neuron degeneration [207]. These mice present with concomitant gradual motor deficits and intraneuronal inclusions, though these inclusions are dissimilar to α-synuclein-containing LBs [207]. This raises an important caveat regarding many genetic models – while it is common to reproduce a single or small subset of core PD features, it has proven difficult to create a mouse model that encompasses all of the pathological aspects of human PD [12,200]. The combination of multiple mutations, or combined treatment with neurotoxins on transgenic backgrounds, are currently being used to better replicate PD-linked pathology and age-dependent dopamine neurodegeneration [210].

More recently, human neurons generated *in vitro* from induced pluripotent stem cells (iPSCs) have risen to prominence as powerful model systems to investigate cellular

mechanisms of dopamine neuron degeneration [45,93,211]. Human dopamine neurons generated from individuals with idiopathic or familial PD can be studied longitudinally – an imperfect but plausible and controllable replication of biological aging – at resolutions previously only possible in animal models or immortalized cell lines [211]. New technology that preserves age-related traits of *in vitro* human neurons by circumventing the reprogramming to pluripotency will potentially enable experiments on human dopamine neurons through aging and neurodegenerative progression [212]. More generally, as probes, imaging methods, animal models, and human *in vitro* preparations continue to advance, subtle changes in synaptic dopamine homeostasis will be trackable in ways that have until now been unachievable. Given the retrograde pattern of degeneration theorized to take place in dopaminergic neurodegeneration, it is possible that a therapeutic intervention targeting synaptic dysfunction may be sufficient to prevent the backpropagation of these processes to the cell body, preserving the longevity of SNc dopamine neurons through aging and disease. These targets are made more promising by the existence of numerous FDA-approved compounds that can target dopamine handling proteins at release sites. Early pathophysiological changes detected at the synaptic level may be more reversible than dysfunction that has reached a cell-wide scale. Longitudinal sub-cellular data on dopamine handling represents potential answers to the critical knowledge gap regarding early processes underlying dopamine neurodegeneration, the closure of which may ultimately reveal therapeutic targets to achieve dopamine neuroprotection in humans.

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References

1. Beeler JA & Dreyer JK Synchronicity: The role of midbrain dopamine in whole brain coordination. eneuro ENEURO.0345-18.2019 (2019). doi:10.1523/ENEURO.0345-18.2019
2. Tian J et al. Distributed and Mixed Information in Monosynaptic Inputs to Dopamine Neurons. Neuron 91, 1374–1389 (2016). [PubMed: 27618675]
3. Lammel S, Lim BK & Malenka RC Reward and aversion in a heterogeneous midbrain dopamine system. Neuropharmacology 76, Part B, 351–359 (2014). [PubMed: 23578393]
4. Watabe-Uchida M, Zhu L, Ogawa SK, Vamanrao A & Uchida N Whole-Brain Mapping of Direct Inputs to Midbrain Dopamine Neurons. Neuron 74, 858–873 (2012). [PubMed: 22681690]
5. Fiorillo CD, Newsome WT & Schultz W The temporal precision of reward prediction in dopamine neurons. Nat. Neurosci 11, 966–973 (2008). [PubMed: 18660807]
6. Cui G et al. Concurrent activation of striatal direct and indirect pathways during action initiation. Nature 494, 238–242 (2013). [PubMed: 23354054]
7. Albin RL, Young AB & Penney JB The functional anatomy of basal ganglia disorders. Trends Neurosci. 12, 366–375 (1989). [PubMed: 2479133]
8. Fu Y et al. A cytoarchitectonic and chemoarchitectonic analysis of the dopamine cell groups in the substantia nigra, ventral tegmental area, and retrorubral field in the mouse. Brain Struct. Funct 217, 591–612 (2012). [PubMed: 21935672]
9. Baik J-H Dopamine Signaling in reward-related behaviors. Front. Neural Circuits 7, (2013).

10. Gibb WR & Lees AJ Anatomy, pigmentation, ventral and dorsal subpopulations of the substantia nigra, and differential cell death in Parkinson's disease. J. Neurol. Neurosurg. Psychiatry 54, 388–396 (1991). [PubMed: 1865199]
11. Hirsch E, Graybiel AM & Agid YA Melanized dopaminergic neurons are differentially susceptible to degeneration in Parkinson's disease. Nature 334, 345–348 (1988). [PubMed: 2899295]
12. Surmeier DJ, Obeso JA & Halliday GM Selective neuronal vulnerability in Parkinson disease. Nat. Rev. Neurosci 18, 101–113 (2017). [PubMed: 28104909]
13. Yin HH et al. Dynamic reorganization of striatal circuits during the acquisition and consolidation of a skill. Nat. Neurosci 12, 333–341 (2009). [PubMed: 19198605]
14. Eisinger RS et al. Medications, Deep Brain Stimulation, and Other Factors Influencing Impulse Control Disorders in Parkinson's Disease. Front. Neurol 10, (2019).
15. Yoo S-W et al. "Depressed" caudate and ventral striatum dopamine transporter availability in de novo Depressed Parkinson's disease. Neurobiol. Dis 104563 (2019). doi:10.1016/j.nbd.2019.104563
16. Colon-Perez LM et al. Cognition and connectomes in nondementia idiopathic Parkinson's disease. Netw. Neurosci 2, 106–124 (2018). [PubMed: 29911667]
17. Yung KKL et al. Immunocytochemical localization of D1 and D2 dopamine receptors in the basal ganglia of the rat: Light and electron microscopy. Neuroscience 65, 709–730 (1995). [PubMed: 7609871]
18. Calipari ES et al. In vivo imaging identifies temporal signature of D1 and D2 medium spiny neurons in cocaine reward. Proc. Natl. Acad. Sci 113, 2726–2731 (2016). [PubMed: 26831103]
19. Gertler TS, Chan CS & Surmeier DJ Dichotomous Anatomical Properties of Adult Striatal Medium Spiny Neurons. J. Neurosci 28, 10814–10824 (2008). [PubMed: 18945889]
20. Bergman H et al. Physiological aspects of information processing in the basal ganglia of normal and parkinsonian primates. Trends Neurosci. 21, 32–38 (1998). [PubMed: 9464684]
21. Beaulieu J-M & Gainetdinov RR The Physiology, Signaling, and Pharmacology of Dopamine Receptors. Pharmacol. Rev 63, 182–217 (2011). [PubMed: 21303898]
22. Ford CP The role of D2-autoreceptors in regulating dopamine neuron activity and transmission. Neuroscience 282, 13–22 (2014). [PubMed: 24463000]
23. Robinson BG et al. Desensitized D2 autoreceptors are resistant to trafficking. Sci. Rep 7, 4379 (2017). [PubMed: 28663556]
24. Wu Q et al. Concurrent Autoreceptor-Mediated Control of Dopamine Release and Uptake during Neurotransmission: An In Vivo Voltammetric Study. J. Neurosci 22, 6272–6281 (2002). [PubMed: 12122086]
25. Lacey MG, Mercuri NB & North RA Dopamine acts on D2 receptors to increase potassium conductance in neurones of the rat substantia nigra zona compacta. J. Physiol 392, 397–416 (1987). [PubMed: 2451725]
26. Beckstead MJ, Ford CP, Phillips PEM & Williams JT Presynaptic regulation of dendrodendritic dopamine transmission. Eur. J. Neurosci 26, 1479–1488 (2007). [PubMed: 17822435]
27. Cragg SJ & Greenfield SA Differential Autoreceptor Control of Somatodendritic and Axon Terminal Dopamine Release in Substantia Nigra, Ventral Tegmental Area, and Striatum. J. Neurosci 17, 5738–5746 (1997). [PubMed: 9221772]
28. Courtney NA, Mamaligas AA & Ford CP Species Differences in Somatodendritic Dopamine Transmission Determine D2-Autoreceptor-Mediated Inhibition of Ventral Tegmental Area Neuron Firing. J. Neurosci 32, 13520–13528 (2012). [PubMed: 23015441]
29. Grace AA Dysregulation of the dopamine system in the pathophysiology of schizophrenia and depression. Nat. Rev. Neurosci 17, 524 (2016). [PubMed: 27256556]
30. Kim H, Andreazza A, Yeung P, Isaacs-Trepanier C & Young LT Oxidation and nitration in dopaminergic areas of the prefrontal cortex from patients with bipolar disorder and schizophrenia. J. Psychiatry Neurosci 39, 276–285 (2014). [PubMed: 24485387]
31. Yang Y et al. Stably maintained microtubules protect dopamine neurons and alleviate depression-like behavior after intracerebral hemorrhage. Sci. Rep 8, 12647 (2018). [PubMed: 30140021]

32. Hansen FH et al. Missense dopamine transporter mutations associate with adult parkinsonism and ADHD. J. Clin. Invest 124, 3107–3120 (2014). [PubMed: 24911152]
33. Bernheimer H, Birkmayer W, Hornykiewicz O, Jellinger K & Seitelberger F Brain dopamine and the syndromes of Parkinson and Huntington Clinical, morphological and neurochemical correlations. J. Neurol. Sci 20, 415–455 (1973). [PubMed: 4272516]
34. Miller DR et al. HIV-1 Tat regulation of dopamine transmission and microglial reactivity is brain region specific. Glia 66, 1915–1928 (2018). [PubMed: 29733459]
35. Kish SJ, Shannak K, Rajput A, Deck JHN & Hornykiewicz O Aging Produces a Specific Pattern of Striatal Dopamine Loss: Implications for the Etiology of Idiopathic Parkinson's Disease. J. Neurochem 58, 642–648 (1992). [PubMed: 1729408]
36. Berg D et al. Time to Redefine PD? Introductory Statement of the MDS Task Force on the Definition of Parkinson's Disease. Mov. Disord 29, 454–462 (2014). [PubMed: 24619848]
37. Dickson DW et al. Evidence that incidental Lewy body disease is pre-symptomatic Parkinson's disease. Acta Neuropathol. (Berl.) 115, 437–444 (2008). [PubMed: 18264713]
38. Braak H et al. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol. Aging 24, 197–211 (2003). [PubMed: 12498954]
39. Parkkinen L, Pirttilä T & Alafuzoff I Applicability of current staging/categorization of α-synuclein pathology and their clinical relevance. Acta Neuropathol. (Berl.) 115, 399–407 (2008). [PubMed: 18297293]
40. Tompkins MM & Hill WD Contribution of somal Lewy bodies to neuronal death. Brain Res. 775, 24–29 (1997). [PubMed: 9439824]
41. Tanaka M et al. Aggresomes Formed by α-Synuclein and Synphilin-1 Are Cytoprotective. J. Biol. Chem 279, 4625–4631 (2004). [PubMed: 14627698]
42. Power JHT, Barnes OL & Chegini F Lewy Bodies and the Mechanisms of Neuronal Cell Death in Parkinson's Disease and Dementia with Lewy Bodies. Brain Pathol. 27, 3–12 (2017). [PubMed: 26667592]
43. Subramaniam M et al. Mutant α-Synuclein Enhances Firing Frequencies in Dopamine Substantia Nigra Neurons by Oxidative Impairment of A-Type Potassium Channels. J. Neurosci 34, 13586–13599 (2014). [PubMed: 25297088]
44. Subramaniam M et al. Selective increase of in vivo firing frequencies in DA SN neurons after proteasome inhibition in the ventral midbrain. Eur. J. Neurosci 40, 2898–2909 (2014). [PubMed: 25059097]
45. Kouroupi G et al. Defective synaptic connectivity and axonal neuropathology in a human iPSC-based model of familial Parkinson's disease. Proc. Natl. Acad. Sci 114, E3679–E3688 (2017). [PubMed: 28416701]
46. Paillusson S et al. α-Synuclein binds to the ER–mitochondria tethering protein VAPB to disrupt Ca2+ homeostasis and mitochondrial ATP production. Acta Neuropathol. (Berl.) 134, 129–149 (2017). [PubMed: 28337542]
47. Ludtmann MHR et al. α-synuclein oligomers interact with ATP synthase and open the permeability transition pore in Parkinson's disease. Nat. Commun 9, 2293 (2018). [PubMed: 29895861]
48. Mosharov EV et al. Interplay between Cytosolic Dopamine, Calcium, and α-Synuclein Causes Selective Death of Substantia Nigra Neurons. Neuron 62, 218–229 (2009). [PubMed: 19409267]
49. Ekstrand MI et al. Progressive parkinsonism in mice with respiratory-chain-deficient dopamine neurons. Proc. Natl. Acad. Sci 104, 1325–1330 (2007). [PubMed: 17227870]
50. Grace AA & Bunney BS The control of firing pattern in nigral dopamine neurons: single spike firing. J. Neurosci 4, 2866–2876 (1984). [PubMed: 6150070]
51. Grace AA & Bunney BS The control of firing pattern in nigral dopamine neurons: burst firing. J. Neurosci 4, 2877–2890 (1984). [PubMed: 6150071]
52. Fiorillo CD, Tobler PN & Schultz W Discrete Coding of Reward Probability and Uncertainty by Dopamine Neurons. Science 299, 1898–1902 (2003). [PubMed: 12649484]
53. Gantz SC, Ford CP, Morikawa H & Williams JT The Evolving Understanding of Dopamine Neurons in the Substantia Nigra and Ventral Tegmental Area. Annu. Rev. Physiol 80, 219–241 (2018). [PubMed: 28938084]

54. Khaliq ZM & Bean BP Pacemaking in Dopaminergic Ventral Tegmental Area Neurons: Depolarizing Drive from Background and Voltage-Dependent Sodium Conductances. J. Neurosci 30, 7401–7413 (2010). [PubMed: 20505107]
55. Khaliq ZM & Bean BP Dynamic, Nonlinear Feedback Regulation of Slow Pacemaking by A-Type Potassium Current in Ventral Tegmental Area Neurons. J. Neurosci 28, 10905–10917 (2008). [PubMed: 18945898]
56. Kang Y & Kitai ST Calcium spike underlying rhythmic firing in dopaminergic neurons of the rat substantia nigra. Neurosci. Res 18, 195–207 (1993). [PubMed: 7907413]
57. Mercuri NB et al. Effects of dihydropyridine calcium antagonists on rat midbrain dopaminergic neurones. Br. J. Pharmacol 113, 831–838 (1994). [PubMed: 7858874]
58. Liss B et al. Tuning pacemaker frequency of individual dopaminergic neurons by Kv4.3L and KChip3.1 transcription. EMBO J. 20, 5715–5724 (2001). [PubMed: 11598014]
59. Krashia P et al. On the properties of identified dopaminergic neurons in the mouse substantia nigra and ventral tegmental area. Eur. J. Neurosci 45, 92–105 (2017). [PubMed: 27519559]
60. Tarfa RA, Evans RC & Khaliq ZM Enhanced Sensitivity to Hyperpolarizing Inhibition in Mesoaccumbal Relative to Nigrostriatal Dopamine Neuron Subpopulations. J. Neurosci 37, 3311–3330 (2017). [PubMed: 28219982]
61. Keath JR, Iacoviello MP, Barrett LE, Mansvelder HD & McGehee DS Differential Modulation by Nicotine of Substantia Nigra Versus Ventral Tegmental Area Dopamine Neurons. J. Neurophysiol 98, 3388–3396 (2007). [PubMed: 17942622]
62. Guzman JN et al. Oxidant stress evoked by pacemaking in dopaminergic neurons is attenuated by DJ-1. Nature 468, 696–700 (2010). [PubMed: 21068725]
63. Guzman JN et al. Systemic isradipine treatment diminishes calcium-dependent mitochondrial oxidant stress. J. Clin. Invest 128, 2266–2280 (2018). [PubMed: 29708514]
64. Lieberman OJ et al. α-Synuclein-Dependent Calcium Entry Underlies Differential Sensitivity of Cultured SN and VTA Dopaminergic Neurons to a Parkinsonian Neurotoxin. eNeuro 4, ENEURO. 0167-17.2017 (2017).
65. Pacelli C et al. Elevated Mitochondrial Bioenergetics and Axonal Arborization Size Are Key Contributors to the Vulnerability of Dopamine Neurons. Curr. Biol 25, 2349–2360 (2015). [PubMed: 26320949]
66. Bender A et al. High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson disease. Nat. Genet 38, 515–517 (2006). [PubMed: 16604074]
67. Bolam JP & Pissadaki EK Living on the edge with too many mouths to feed: Why dopamine neurons die. Mov. Disord 27, 1478–1483 (2012). [PubMed: 23008164]
68. Dopeso-Reyes IG et al. Calbindin content and differential vulnerability of midbrain efferent dopaminergic neurons in macaques. Front. Neuroanat 8, (2014).
69. Poulin J-F et al. Defining Midbrain Dopaminergic Neuron Diversity by Single-Cell Gene Expression Profiling. Cell Rep. 9, 930–943 (2014). [PubMed: 25437550]
70. Yamada T, McGeer PL, Baimbridge KG & McGeer EG Relative sparing in Parkinson's disease of substantia nigra dopamine neurons containing calbindin-D28K. Brain Res. 526, 303–307 (1990). [PubMed: 2257487]
71. Liang C-L, Sinton CM, Sonsalla PK & German DC Midbrain Dopaminergic Neurons in the Mouse that Contain Calbindin-D28kExhibit Reduced Vulnerability to MPTP-induced Neurodegeneration. Neurodegeneration 5, 313–318 (1996). [PubMed: 9117542]
72. Hage TA & Khaliq ZM Tonic Firing Rate Controls Dendritic Ca2+ Signaling and Synaptic Gain in Substantia Nigra Dopamine Neurons. J. Neurosci 35, 5823–5836 (2015). [PubMed: 25855191]
73. Matsuda W et al. Single Nigrostriatal Dopaminergic Neurons Form Widely Spread and Highly Dense Axonal Arborizations in the Neostriatum. J. Neurosci 29, 444–453 (2009). [PubMed: 19144844]
74. Chu Y & Kordower JH Age-associated increases of α-synuclein in monkeys and humans are associated with nigrostriatal dopamine depletion: Is this the target for Parkinson's disease? Neurobiol. Dis 25, 134–149 (2007). [PubMed: 17055279]

75. Descarries L, Watkins KC, Garcia S, Bosler O & Doucet G Dual character, asynaptic and synaptic, of the dopamine innervation in adult rat neostriatum: A quantitative autoradiographic and immunocytochemical analysis. J. Comp. Neurol 375, 167–186 (1996). [PubMed: 8915824]
76. Descarries L & Mechawar N Ultrastructural evidence for diffuse transmission by monoamine and acetylcholine neurons of the central nervous system in Progress in Brain Research 125, 27–47 (Elsevier, 2000). [PubMed: 11098652]
77. Silm K et al. Synaptic Vesicle Recycling Pathway Determines Neurotransmitter Content and Release Properties. Neuron (2019). doi:10.1016/j.neuron.2019.03.031
78. Liu C, Kershberg L, Wang J, Schneeberger S & Kaeser PS Dopamine Secretion Is Mediated by Sparse Active Zone-like Release Sites. Cell 172, 706–718.e15 (2018). [PubMed: 29398114]
79. Cragg SJ & Rice ME DAncing past the DAT at a DA synapse. Trends Neurosci. 27, 270–277 (2004). [PubMed: 15111009]
80. Pan P-Y & Ryan TA Calbindin controls release probability in ventral tegmental area dopamine neurons. Nat. Neurosci 15, 813–815 (2012). [PubMed: 22544312]
81. Pereira DB et al. Fluorescent false neurotransmitter reveals functionally silent dopamine vesicle clusters in the striatum. Nat. Neurosci 19, 578–586 (2016). [PubMed: 26900925]
82. Ewing AG, Bigelow JC & Wightman RM Direct in vivo monitoring of dopamine released from two striatal compartments in the rat. Science 221, 169–171 (1983). [PubMed: 6857277]
83. Covey DP, Juliano SA & Garris PA Amphetamine Elicits Opposing Actions on Readily Releasable and Reserve Pools for Dopamine. PLOS ONE 8, e60763 (2013). [PubMed: 23671560]
84. Cheng H-C, Ulane CM & Burke RE Clinical progression in Parkinson disease and the neurobiology of axons. Ann. Neurol 67, 715–725 (2010). [PubMed: 20517933]
85. Tagliaferro P & Burke RE Retrograde Axonal Degeneration in Parkinson Disease. J. Park. Dis 6, 1–15 (2016).
86. Caminiti SP et al. Axonal damage and loss of connectivity in nigrostriatal and mesolimbic dopamine pathways in early Parkinson's disease. NeuroImage Clin. 14, 734–740 (2017). [PubMed: 28409113]
87. Scott DA et al. A Pathologic Cascade Leading to Synaptic Dysfunction in α-Synuclein-Induced Neurodegeneration. J. Neurosci 30, 8083–8095 (2010). [PubMed: 20554859]
88. Braak H & Del Tredici K Poor and protracted myelination as a contributory factor to neurodegenerative disorders. Neurobiol. Aging 25, 19–23 (2004). [PubMed: 14675725]
89. Braak H, Rüb U, Gai WP & Del Tredici K Idiopathic Parkinson's disease: possible routes by which vulnerable neuronal types may be subject to neuroinvasion by an unknown pathogen. J. Neural Transm 110, 517–536 (2003). [PubMed: 12721813]
90. Pissadaki EK & Bolam JP The energy cost of action potential propagation in dopamine neurons: clues to susceptibility in Parkinson's disease. Front. Comput. Neurosci 7, (2013).
91. Berthet A et al. Loss of Mitochondrial Fission Depletes Axonal Mitochondria in Midbrain Dopamine Neurons. J. Neurosci 34, 14304–14317 (2014). [PubMed: 25339743]
92. Deng H, Dodson MW, Huang H & Guo M The Parkinson's disease genes pink1 and parkin promote mitochondrial fission and/or inhibit fusion in Drosophila. Proc. Natl. Acad. Sci 105, 14503–14508 (2008). [PubMed: 18799731]
93. Chung SY et al. Parkin and PINK1 Patient iPSC-Derived Midbrain Dopamine Neurons Exhibit Mitochondrial Dysfunction and α-Synuclein Accumulation. Stem Cell Rep. 7, 664–677 (2016).
94. Buhlman LM Parkin loss-of-function pathology: Premature neuronal senescence induced by high levels of reactive oxygen species? Mech. Ageing Dev 161, 112–120 (2017). [PubMed: 27374431]
95. Volpicelli-Daley LA et al. Exogenous α-Synuclein Fibrils Induce Lewy Body Pathology Leading to Synaptic Dysfunction and Neuron Death. Neuron 72, 57–71 (2011). [PubMed: 21982369]
96. Wu Q et al. Alpha-synuclein (αSyn) preformed fibrils induce endogenous αSyn aggregation, compromise synaptic activity and enhance synapse loss in cultured excitatory hippocampal neurons. J. Neurosci 0060–19 (2019). doi:10.1523/JNEUROSCI.0060-19.2019
97. Pranke IM et al. α-Synuclein and ALPS motifs are membrane curvature sensors whose contrasting chemistry mediates selective vesicle binding. J. Cell Biol 194, 89–103 (2011). [PubMed: 21746853]

98. Middleton ER & Rhoades E Effects of Curvature and Composition on α-Synuclein Binding to Lipid Vesicles. Biophys. J 99, 2279–2288 (2010). [PubMed: 20923663]

99. Sulzer D & Edwards RH The physiological role of α-synuclein and its relationship to Parkinson's Disease. J. Neurochem 150, 475–486 (2019). [PubMed: 31269263]

100. Logan T, Bendor J, Toupin C, Thorn K & Edwards RH α-Synuclein promotes dilation of the exocytotic fusion pore. Nat. Neurosci 20, 681–689 (2017). [PubMed: 28288128]

101. Abbineni PS, Bohannon KP, Bittner MA, Axelrod D & Holz RW Identification of β-synuclein on secretory granules in chromaffin cells and the effects of α- and β-synuclein on post-fusion BDNF discharge and fusion pore expansion. Neurosci. Lett 699, 134–139 (2019). [PubMed: 30711526]

102. Burré J, Sharma M & Südhof TC α-Synuclein assembles into higher-order multimers upon membrane binding to promote SNARE complex formation. Proc. Natl. Acad. Sci 111, E4274–E4283 (2014). [PubMed: 25246573]

103. Garcia-Reitböck P et al. SNARE protein redistribution and synaptic failure in a transgenic mouse model of Parkinson's disease. Brain 133, 2032–2044 (2010). [PubMed: 20534649]

104. Busch DJ et al. Acute increase of α-synuclein inhibits synaptic vesicle recycling evoked during intense stimulation. Mol. Biol. Cell 25, 3926–3941 (2014). [PubMed: 25273557]

105. Graham DG Oxidative Pathways for Catecholamines in the Genesis of Neuromelanin and Cytotoxic Quinones. Mol. Pharmacol 14, 633–643 (1978). [PubMed: 98706]

106. Chen L et al. Unregulated Cytosolic Dopamine Causes Neurodegeneration Associated with Oxidative Stress in Mice. J. Neurosci 28, 425–433 (2008). [PubMed: 18184785]

107. Zhang S, Wang R & Wang G Impact of dopamine oxidation on dopaminergic neurodegeneration. ACS Chem. Neurosci (2018). doi:10.1021/acschemneuro.8b00454

108. Torres GE, Gainetdinov RR & Caron MG Plasma membrane monoamine transporters: structure, regulation and function. Nat. Rev. Neurosci 4, 13–25 (2003). [PubMed: 12511858]

109. Cass WA, Gerhardt GA, Mayfield RD, Curella P & Zahniser NR Differences in Dopamine Clearance and Diffusion in Rat Striatum and Nucleus Accumbens Following Systemic Cocaine Administration. J. Neurochem 59, 259–266 (1992). [PubMed: 1613502]

110. Khoshbouei H, Wang H, Lechleiter JD, Javitch JA & Galli A Amphetamine-induced Dopamine Efflux A VOLTAGE-SENSITIVE AND INTRACELLULAR Na+-DEPENDENT MECHANISM. J. Biol. Chem 278, 12070–12077 (2003). [PubMed: 12556446]

111. Sambo DO et al. The sigma-1 receptor modulates methamphetamine dysregulation of dopamine neurotransmission. Nat. Commun 8, 2228 (2017). [PubMed: 29263318]

112. Freyberg Z et al. Mechanisms of amphetamine action illuminated through optical monitoring of dopamine synaptic vesicles in Drosophila brain. Nat. Commun 7, 10652 (2016). [PubMed: 26879809]

113. Rahbek-Clemmensen T et al. Super-resolution microscopy reveals functional organization of dopamine transporters into cholesterol and neuronal activity-dependent nanodomains. Nat. Commun 8, 740 (2017). [PubMed: 28963530]

114. Chen BT & Rice ME Novel Ca2+ Dependence and Time Course of Somatodendritic Dopamine Release: Substantia Nigra versus Striatum. J. Neurosci 21, 7841–7847 (2001). [PubMed: 11567075]

115. Falkenburger BH, Barstow KL & Mintz IM Dendrodendritic Inhibition Through Reversal of Dopamine Transport. Science 293, 2465–2470 (2001). [PubMed: 11577238]

116. Rice Margaret E & Patel Jyoti C Somatodendritic dopamine release: recent mechanistic insights. Philos. Trans. R. Soc. B Biol. Sci 370, 20140185 (2015).

117. Robinson BG et al. RIM is essential for stimulated but not spontaneous somatodendritic dopamine release in the midbrain. eLife 8, e47972 (2019). [PubMed: 31486769]

118. Kurian MA et al. Homozygous loss-of-function mutations in the gene encoding the dopamine transporter are associated with infantile parkinsonism-dystonia. J. Clin. Invest 119, 1595–1603 (2009). [PubMed: 19478460]

119. Campbell NG et al. Structural, functional, and behavioral insights of dopamine dysfunction revealed by a deletion in SLC6A3. Proc. Natl. Acad. Sci 116, 3853–3862 (2019). [PubMed: 30755521]

120. Ng J et al. Dopamine transporter deficiency syndrome: phenotypic spectrum from infancy to adulthood. Brain 137, 1107–1119 (2014). [PubMed: 24613933]

121. Sakrikar D et al. Attention Deficit/Hyperactivity Disorder-Derived Coding Variation in the Dopamine Transporter Disrupts Microdomain Targeting and Trafficking Regulation. J. Neurosci 32, 5385–5397 (2012). [PubMed: 22514303]

122. Mazei-Robison MS et al. Anomalous Dopamine Release Associated with a Human Dopamine Transporter Coding Variant. J. Neurosci 28, 7040–7046 (2008). [PubMed: 18614672]

123. Kurian MA et al. Clinical and molecular characterisation of hereditary dopamine transporter deficiency syndrome: an observational cohort and experimental study. Lancet Neurol. 10, 54–62 (2011). [PubMed: 21112253]

124. Cyr M et al. Sustained elevation of extracellular dopamine causes motor dysfunction and selective degeneration of striatal GABAergic neurons. Proc. Natl. Acad. Sci 100, 11035–11040 (2003). [PubMed: 12958210]

125. Giros B, Jaber M, Jones SR, Wightman RM & Caron MG Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. Nature 379, 606–612 (1996). [PubMed: 8628395]

126. Bezard E et al. Absence of MPTP-Induced Neuronal Death in Mice Lacking the Dopamine Transporter. Exp. Neurol 155, 268–273 (1999). [PubMed: 10072302]

127. Javitch JA, D'Amato RJ, Strittmatter SM & Snyder SH Parkinsonism-inducing neurotoxin, N-methyl-4-phenyl-1,2,3,6 -tetrahydropyridine: uptake of the metabolite N-methyl-4-phenylpyridine by dopamine neurons explains selective toxicity. Proc. Natl. Acad. Sci. U. S. A 82, 2173–2177 (1985). [PubMed: 3872460]

128. Stamford JA, Kruk ZL, Palij P & Millar J Diffusion and uptake of dopamine in rat caudate and nucleus accumbens compared using fast cyclic voltammetry. Brain Res. 448, 381–385 (1988). [PubMed: 3378163]

129. Suaud-Chagny MF, Dugast C, Chergui K, Msghina M & Gonon F Uptake of Dopamine Released by Impulse Flow in the Rat Mesolimbic and Striatal Systems In Vivo. J. Neurochem 65, 2603–2611 (1995). [PubMed: 7595557]

130. Langston JW, Ballard P, Tetrud JW & Irwin I Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. Science 219, 979–980 (1983). [PubMed: 6823561]

131. Dorsey ER et al. Global, regional, and national burden of Parkinson's disease, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol. 17, 939–953 (2018). [PubMed: 30287051]

132. Van Maele-Fabry G, Hoet P, Vilain F & Lison D Occupational exposure to pesticides and Parkinson's disease: A systematic review and meta-analysis of cohort studies. Environ. Int 46, 30–43 (2012). [PubMed: 22698719]

133. Pearson J, Goldstein M & Brandeis L Tyrosine hydroxylase immunohistochemistry in human brain. Brain Res. 165, 333–337 (1979). [PubMed: 33748]

134. Gaspar P et al. Tyrosine hydroxylase and methionine-enkephalin in the human mesencephalon: Immunocytochemical localization and relationships. J. Neurol. Sci 58, 247–267 (1983). [PubMed: 6131945]

135. Pickel VM, Beckley SC, Joh TH & Reis DJ Ultrastructural immunocytochemical localization of tyrosine hydroxylase in the neostriatum. Brain Res. 225, 373–385 (1981). [PubMed: 6118197]

136. Reyes S et al. GIRK2 expression in dopamine neurons of the substantia nigra and ventral tegmental area. J. Comp. Neurol 520, 2591–2607 (2012). [PubMed: 22252428]

137. Kordower JH et al. Disease duration and the integrity of the nigrostriatal system in Parkinson's disease. Brain 136, 2419–2431 (2013). [PubMed: 23884810]

138. Sesack SR, Snyder CL & Lewis DA Axon terminals immunolabeled for dopamine or tyrosine hydroxylase synapse on GABA-immunoreactive dendrites in rat and monkey cortex. J. Comp. Neurol 363, 264–280 (1995). [PubMed: 8642074]

139. Lyng GD, Snyder-Keller A & Seegal RF Dopaminergic Development of Prenatal Ventral Mesencephalon and Striatum in Organotypic Co-Cultures. Brain Res. 1133, 1–9 (2007). [PubMed: 17196555]

140. Ford CP, Gantz SC, Phillips PEM & Williams JT Control of Extracellular Dopamine at Dendrite and Axon Terminals. J. Neurosci 30, 6975–6983 (2010). [PubMed: 20484639]
141. Salvatore MF & Pruett BS Dichotomy of Tyrosine Hydroxylase and Dopamine Regulation between Somatodendritic and Terminal Field Areas of Nigrostriatal and Mesoaccumbens Pathways. PLOS ONE 7, e29867 (2012). [PubMed: 22242182]
142. Grattan-Smith PJ et al. Tyrosine hydroxylase deficiency: Clinical manifestations of catecholamine insufficiency in infancy. Mov. Disord 17, 354–359 (2002). [PubMed: 11921123]
143. Willemsen MA et al. Tyrosine hydroxylase deficiency: a treatable disorder of brain catecholamine biosynthesis. Brain 133, 1810–1822 (2010). [PubMed: 20430833]
144. Zhou Q-Y, Quaife CJ & Palmiter RD Targeted disruption of the tyrosine hydroxylase gene reveals that catecholamines are required for mouse fetal development. Nature 374, 640 (1995). [PubMed: 7715703]
145. Kobayashi K et al. Targeted Disruption of the Tyrosine Hydroxylase Locus Results in Severe Catecholamine Depletion and Perinatal Lethality in Mice. J. Biol. Chem 270, 27235–27243 (1995). [PubMed: 7592982]
146. Althini S et al. Normal nigrostriatal innervation but dopamine dysfunction in mice carrying hypomorphic tyrosine hydroxylase alleles. J. Neurosci. Res 72, 444–453 (2003). [PubMed: 12704806]
147. Zauli G et al. HIV-1 Tat-mediated Inhibition of the Tyrosine Hydroxylase Gene Expression in Dopaminergic Neuronal Cells. J. Biol. Chem 275, 4159–4165 (2000). [PubMed: 10660577]
148. Eiden LE & Weihe E VMAT2: a dynamic regulator of brain monoaminergic neuronal function interacting with drugs of abuse. Ann. N. Y. Acad. Sci 1216, 86–98 (2011). [PubMed: 21272013]
149. Nirenberg MJ, Chan J, Liu Y, Edwards RH & Pickel VM Ultrastructural Localization of the Vesicular Monoamine Transporter-2 in Midbrain Dopaminergic Neurons: Potential Sites for Somatodendritic Storage and Release of Dopamine. J. Neurosci 16, 4135–4145 (1996). [PubMed: 8753875]
150. Nirenberg MJ, Chan J, Liu Y, Edwards RH & Pickel VM Vesicular monoamine transporter-2: Immunogold localization in striatal axons and terminals. Synapse 26, 194–198 (1997). [PubMed: 9131778]
151. Rilstone JJ, Alkhater RA & Minassian BA Brain Dopamine–Serotonin Vesicular Transport Disease and Its Treatment. 10.1056/NEJMoa1207281 (2013). doi:10.1056/NEJMoa1207281
152. Patel N et al. Genetic investigation of 93 families with microphthalmia or posterior microphthalmos. Clin. Genet 93, 1210–1222 (2018). [PubMed: 29450879]
153. Burman J, Tran CH, Glatt C, Freimer NB & Edwards RH The effect of rare human sequence variants on the function of vesicular monoamine transporter 2. Pharmacogenet. Genomics 14, 587 (2004).
154. Fon EA et al. Vesicular Transport Regulates Monoamine Storage and Release but Is Not Essential for Amphetamine Action. Neuron 19, 1271–1283 (1997). [PubMed: 9427250]
155. Wang Y-M et al. Knockout of the Vesicular Monoamine Transporter 2 Gene Results in Neonatal Death and Supersensitivity to Cocaine and Amphetamine. Neuron 19, 1285–1296 (1997). [PubMed: 9427251]
156. Takahashi N et al. VMAT2 knockout mice: Heterozygotes display reduced amphetamine-conditioned reward, enhanced amphetamine locomotion, and enhanced MPTP toxicity. Proc. Natl. Acad. Sci 94, 9938–9943 (1997). [PubMed: 9275230]
157. Caudle WM et al. Reduced Vesicular Storage of Dopamine Causes Progressive Nigrostriatal Neurodegeneration. J. Neurosci 27, 8138–8148 (2007). [PubMed: 17652604]
158. Steinkellner T et al. Role for VGLUT2 in selective vulnerability of midbrain dopamine neurons. J. Clin. Invest 128, 774–788 (2018). [PubMed: 29337309]
159. Aghajanian GK & Bunney BS Dopamine “Autoreceptors”: Pharmacological characterization by microiontophoretic single cell recording studies. Naunyn. Schmiedebergs Arch. Pharmacol 297, 1–7 (1977). [PubMed: 193046]
160. Giros B et al. Alternative splicing directs the expression of two D 2 dopamine receptor isoforms. Nature 342, 923 (1989). [PubMed: 2531847]

161. Usiello A et al. Distinct functions of the two isoforms of dopamine D2 receptors. Nature 408, 199 (2000). [PubMed: 11089973]
162. Boulay D, Depoortere R, Perrault G, Borrelli E & Sanger DJ Dopamine D2 receptor knock-out mice are insensitive to the hypolocomotor and hypothermic effects of dopamine D2/D3 receptor agonists. Neuropharmacology 38, 1389–1396 (1999). [PubMed: 10471093]
163. Beckstead MJ, Grandy DK, Wickman K & Williams JT Vesicular Dopamine Release Elicits an Inhibitory Postsynaptic Current in Midbrain Dopamine Neurons. Neuron 42, 939–946 (2004). [PubMed: 15207238]
164. Perra S, Clements MA, Bernier BE & Morikawa H In Vivo Ethanol Experience Increases D2 Autoinhibition in the Ventral Tegmental Area. Neuropsychopharmacology 36, 993–1002 (2011). [PubMed: 21248720]
165. Richardson BD et al. Membrane potential shapes regulation of dopamine transporter trafficking at the plasma membrane. Nat. Commun 7, 10423 (2016). [PubMed: 26804245]
166. Benoit-Marand M, Ballion B, Borrelli E, Boraud T & Gonon F Inhibition of dopamine uptake by D2 antagonists: an in vivo study. J. Neurochem 116, 449–458 (2011). [PubMed: 21128941]
167. Mayfield RD & Zahniser NR Dopamine D2 Receptor Regulation of the Dopamine Transporter Expressed in Xenopus laevis Oocytes Is Voltage-Independent. Mol. Pharmacol 59, 113–121 (2001). [PubMed: 11125031]
168. Lee FJ et al. Dopamine transporter cell surface localization facilitated by a direct interaction with the dopamine D2 receptor. EMBO J. 26, 2127–2136 (2007). [PubMed: 17380124]
169. Bolan EA et al. D2 Receptors Regulate Dopamine Transporter Function via an Extracellular Signal-Regulated Kinases 1 and 2-Dependent and Phosphoinositide 3 Kinase-Independent Mechanism. Mol. Pharmacol 71, 1222–1232 (2007). [PubMed: 17267664]
170. Cardozo DL & Bean BP Voltage-dependent calcium channels in rat midbrain dopamine neurons: modulation by dopamine and GABAB receptors. J. Neurophysiol 74, 1137–1148 (1995). [PubMed: 7500139]
171. Wang J & Sampson S Sulpiride versus placebo for schizophrenia. Cochrane Database Syst. Rev (2014). doi:10.1002/14651858.CD007811.pub2
172. Rice ME & Cragg SJ Dopamine spillover after quantal release: Rethinking dopamine transmission in the nigrostriatal pathway. Brain Res. Rev 58, 303–313 (2008). [PubMed: 18433875]
173. Lammel S et al. Unique Properties of Mesoprefrontal Neurons within a Dual Mesocorticolimbic Dopamine System. Neuron 57, 760–773 (2008). [PubMed: 18341995]
174. Iravani MM, Muscat R & Kruk ZL Comparison of somatodendritic and axon terminal dopamine release in the ventral tegmental area and the nucleus accumbens. Neuroscience 70, 1025–1037 (1996). [PubMed: 8848165]
175. Li X, Qi J, Yamaguchi T, Wang H-L & Morales M Heterogeneous composition of dopamine neurons of the rat A10 region: molecular evidence for diverse signaling properties. Brain Struct. Funct 218, 1159–1176 (2013). [PubMed: 22926514]
176. Thompson L Identification of Dopaminergic Neurons of Nigral and Ventral Tegmental Area Subtypes in Grafts of Fetal Ventral Mesencephalon Based on Cell Morphology, Protein Expression, and Efferent Projections. J. Neurosci 25, 6467–6477 (2005). [PubMed: 16000637]
177. Di Salvio M et al. Otx2 controls neuron subtype identity in ventral tegmental area and antagonizes vulnerability to MPTP. Nat. Neurosci 13, 1481–1488 (2010). [PubMed: 21057506]
178. Inanobe A et al. Characterization of G-Protein-Gated K+ Channels Composed of Kir3.2 Subunits in Dopaminergic Neurons of the Substantia Nigra. J. Neurosci 19, 1006–1017 (1999). [PubMed: 9920664]
179. Cruz HG et al. Bi-directional effects of GABA B receptor agonists on the mesolimbic dopamine system. Nat. Neurosci 7, 153 (2004). [PubMed: 14745451]
180. Munoz MB et al. A Role for the GIRK3 Subunit in Methamphetamine-Induced Attenuation of GABAB Receptor-Activated GIRK Currents in VTA Dopamine Neurons. J. Neurosci 36, 3106–3114 (2016). [PubMed: 26985023]

181. Kehr W, Carlsson A, Lindqvist M, Magnusson T & Atack C Evidence for a receptor-mediated feedback control of striatal tyrosine hydroxylase activity. J. Pharm. Pharmacol 24, 744–747 (1972). [PubMed: 4404084]
182. Anzalone A et al. Dual Control of Dopamine Synthesis and Release by Presynaptic and Postsynaptic Dopamine D2 Receptors. J. Neurosci 32, 9023–9034 (2012). [PubMed: 22745501]
183. Ashe HL & Briscoe J The interpretation of morphogen gradients. Development 133, 385–394 (2006). [PubMed: 16410409]
184. Blaess S & Ang S-L Genetic control of midbrain dopaminergic neuron development. Wiley Interdiscip. Rev. Dev. Biol 4, 113–134 [PubMed: 25565353]
185. Veenvliet JV & Smidt MP Molecular mechanisms of dopaminergic subset specification: fundamental aspects and clinical perspectives. Cell. Mol. Life Sci 71, 4703–4727 (2014). [PubMed: 25064061]
186. Liu G et al. Aldehyde dehydrogenase 1 defines and protects a nigrostriatal dopaminergic neuron subpopulation. J. Clin. Invest 124, 3032–3046 (2014). [PubMed: 24865427]
187. Doucet-Beaupré H & Lévesque M The role of developmental transcription factors in adult midbrain dopaminergic neurons. OA Neurosci. 1, (2013).
188. Damier P, Hirsch EC, Agid Y & Graybiel AM The substantia nigra of the human brainII. Patterns of loss of dopamine-containing neurons in Parkinson's disease. Brain 122, 1437–1448 (1999). [PubMed: 10430830]
189. 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 Chem. Neurosci 4, 858–869 (2013). [PubMed: 23647019]
190. Patriarchi T et al. Ultrafast neuronal imaging of dopamine dynamics with designed genetically encoded sensors. Science eaat4422 (2018). doi:10.1126/science.aat4422 [PubMed: 29853555]
191. Sun F et al. A Genetically Encoded Fluorescent Sensor Enables Rapid and Specific Detection of Dopamine in Flies, Fish, and Mice. Cell 174, 481–496.e19 (2018). [PubMed: 30007419]
192. Eriksen J et al. Visualization of Dopamine Transporter Trafficking in Live Neurons by Use of Fluorescent Cocaine Analogs. J. Neurosci 29, 6794–6808 (2009). [PubMed: 19474307]
193. Bezard E, Dovero S, Bioulac B & Gross C Effects of Different Schedules of MPTP Administration on Dopaminergic Neurodegeneration in Mice. Exp. Neurol 148, 288–292 (1997). [PubMed: 9398471]
194. Alvarez-Fischer D et al. Characterization of the striatal 6-OHDA model of Parkinson's disease in wild type and α-synuclein-deleted mice. Exp. Neurol 210, 182–193 (2008). [PubMed: 18053987]
195. Porras G, Li Q & Bezard E Modeling Parkinson's Disease in Primates: The MPTP Model. Cold Spring Harb. Perspect. Med 2, (2012).
196. Willard AM, Bouchard RS & Gittis AH Differential degradation of motor deficits during gradual dopamine depletion with 6-hydroxydopamine in mice. Neuroscience 301, 254–267 (2015). [PubMed: 26067595]
197. Redman PT et al. A vital role for voltage-dependent potassium channels in dopamine transporter-mediated 6-hydroxydopamine neurotoxicity. Neuroscience 143, 1–6 (2006). [PubMed: 17027171]
198. Naudet N et al. Oral Exposure to Paraquat Triggers Earlier Expression of Phosphorylated α-Synuclein in the Enteric Nervous System of A53T Mutant Human α-Synuclein Transgenic Mice. J. Neuropathol. Exp. Neurol 76, 1046–1057 (2017). [PubMed: 29040593]
199. Kim S et al. Transneuronal Propagation of Pathologic α-Synuclein from the Gut to the Brain Models Parkinson's Disease. Neuron 0, (2019).
200. Raza C, Anjum R & Shakeel N ul A. Parkinson's disease: Mechanisms, translational models and management strategies. Life Sci. 226, 77–90 (2019). [PubMed: 30980848]
201. Dauer W & Przedborski S Parkinson's Disease: Mechanisms and Models. Neuron 39, 889–909 (2003). [PubMed: 12971891]
202. Janus C & Welzl H Mouse Models of Neurodegenerative Diseases: Criteria and General Methodology in Mouse Models for Drug Discovery (eds. Proetzel G & Wiles MV) 323–345 (Humana Press, 2010). doi:10.1007/978-1-60761-058-8_19

203. Wong PC, Cai H, Borchelt DR & Price DL Genetically engineered mouse models of neurodegenerative diseases. Nat. Neurosci 5, 633–639 (2002). [PubMed: 12085093]

204. Parrado-Fernández C et al. Reduction of PINK1 or DJ-1 impair mitochondrial motility in neurites and alter ER-mitochondria contacts. J. Cell. Mol. Med 0,

205. Ip CW et al. AAV1/2-induced overexpression of A53T-α-synuclein in the substantia nigra results in degeneration of the nigrostriatal system with Lewy-like pathology and motor impairment: a new mouse model for Parkinson's disease. Acta Neuropathol. Commun 5, 11 (2017). [PubMed: 28143577]

206. Branch SY et al. Dopaminergic Neurons Exhibit an Age-Dependent Decline in Electrophysiological Parameters in the MitoPark Mouse Model of Parkinson's Disease. J. Neurosci 36, 4026–4037 (2016). [PubMed: 27053209]

207. Ekstrand MI & Galter D The MitoPark Mouse – An animal model of Parkinson's disease with impaired respiratory chain function in dopamine neurons. Parkinsonism Relat. Disord 15, Supplement 3, S185–S188 (2009). [PubMed: 20082987]

208. Farrand AQ, Gregory RA, Bäckman CM, Helke KL & Boger HA Altered glutamate release in the dorsal striatum of the MitoPark mouse model of Parkinson's disease. Brain Res. 1651, 88–94 (2016). [PubMed: 27659966]

209. Ghaisas S et al. MitoPark Transgenic Mouse Model Recapitulates the Gastrointestinal Dysfunction and Gut-Microbiome Changes of Parkinson's Disease. NeuroToxicology (2019). doi:10.1016/j.neuro.2019.09.004

210. Sliter DA et al. Parkin and PINK1 mitigate STING-induced inflammation. Nature 561, 258–262 (2018). [PubMed: 30135585]

211. Burbulla LF et al. Dopamine oxidation mediates mitochondrial and lysosomal dysfunction in Parkinson's disease. Science 357, 1255–1261 (2017). [PubMed: 28882997]

212. Kim Y et al. Mitochondrial Aging Defects Emerge in Directly Reprogrammed Human Neurons due to Their Metabolic Profile. Cell Rep. 23, 2550–2558 (2018). [PubMed: 29847787]

Highlights

- Preventing age-dependent dopaminergic neurodegeneration remains an unmet challenge
- Degenerative damage appears to originate at dopamine release sites
- Release sites are phenotypically heterogenous in both structure and function
- Synaptic phenotypes correlate with degeneration and may reveal therapeutic targets

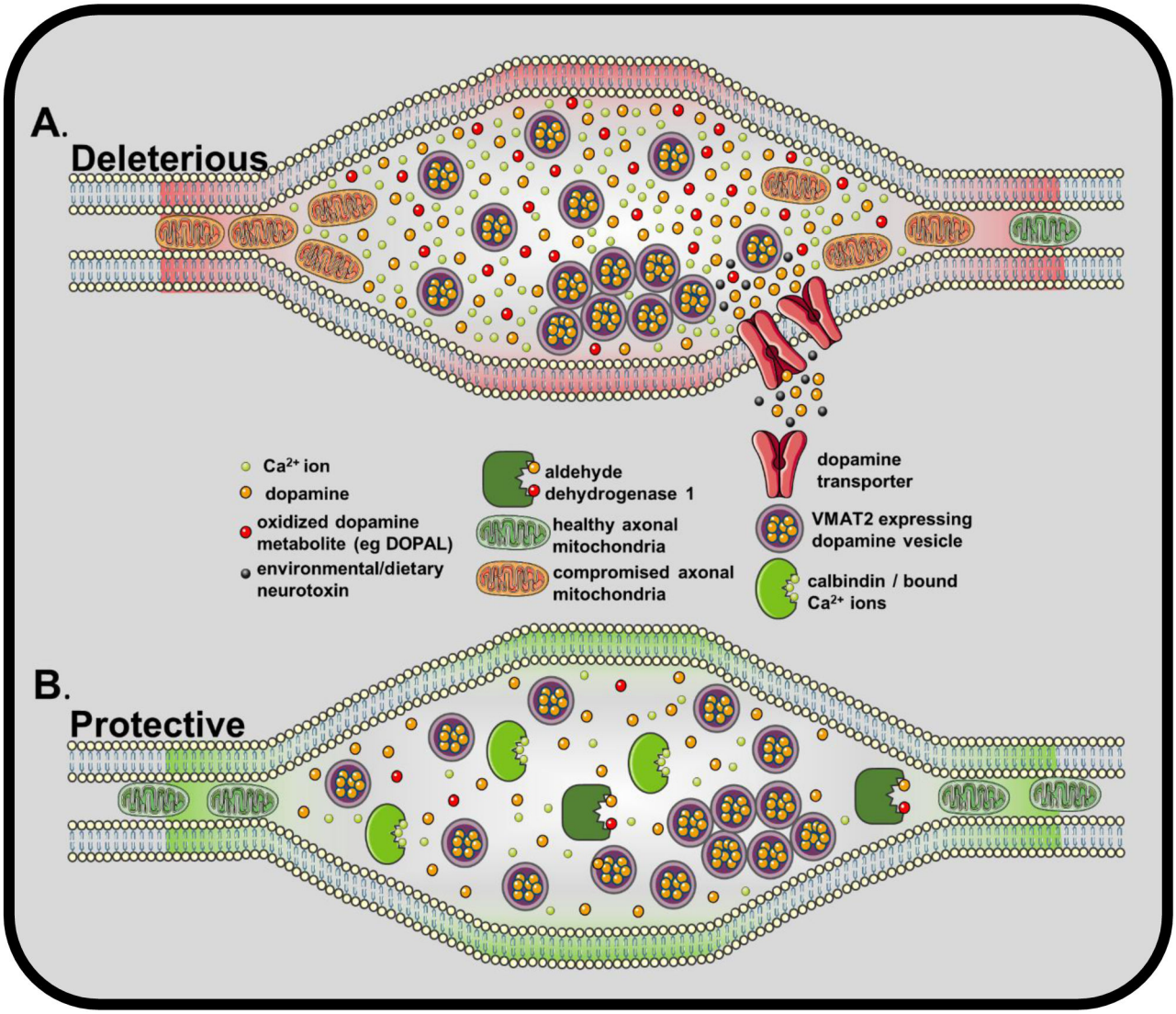

Figure 1. protective and deleterious elements of dopamine release sites.

A. Schematic of a release site in terminal regions of dopamine neurons. Release sites are flanked by unmyelinated axonal compartments, which harbor pools of axonal mitochondria. Repeated exposure to large increases in intracellular Ca^{2+} ions induce mitochondrial damage, leading to compromised bioenergetics. Cytosolic dopamine (orange) is hypothesized to be higher in disease sensitive neurons than non-sensitive counterparts, resulting in increased levels of oxidized dopamine metabolites (red) capable of causing damage to intracellular and transmembrane components. Additionally, dopamine transporters at release sites may uptake environmental and dietary neurotoxins at a level correlated with transporter expression. **B.** The Ca^{2+} binding protein calbindin, coupled with less reliance on Ca^{2+} for pacemaking, limits damage to axonal mitochondria preventing bioenergetic failure. Aldehyde dehydrogenase – an enzyme the converts aldehyde groups to carboxylic acid – acts on selected dopamine oxidation metabolites including dopamine -3,4,-

dihydroxyphenylacetaldehyde (DOPAL) to reduce ROS generation and subsequent cell damage.