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Alpha-synuclein modulates dopamine neurotransmission

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Abstract

Alpha-synuclein is a small, highly charged protein encoded by the synuclein or *SNCA* gene that is predominantly expressed in central nervous system neurons. Although its physiological function remains enigmatic, alpha-synuclein is implicated in movement disorders such as Parkinson's disease, multiple system atrophy, and in neurodegenerative diseases such as Dementia with Lewy bodies. Here we have focused on reviewing the existing literature pertaining to wild-type alpha-synuclein structure, its properties, and its potential involvement in regulation of dopamine neurotransmission.

Introduction

Alpha-synuclein is a small, highly charged protein encoded by synuclein or *SNCA* gene that is predominantly expressed in central nervous system (CNS) neurons [3, 4]. The function of alpha-synuclein under both physiological and pathological conditions remains elusive. Alpha-synuclein is implicated in movement disorders such as Parkinson's disease (PD), multiple system atrophy (MSA), and in neurodegenerative diseases such as Dementia with Lewy bodies (DLB) [7]. A hallmark of PD is a significant loss of dopaminergic neurons in the substantia nigra pars compacta, with some of the remaining neurons containing alpha-synuclein positive inclusions known as Lewy Bodies. Missense alpha-synuclein point mutations, as well as duplication and triplication of the synuclein gene, have been implicated in familial PD resulting in rapid and early onset of classical symptoms [10, 11]. Postmortem human studies show increased levels of alpha-synuclein in ventral tegmental area (VTA) and substantia nigra dopaminergic neurons of cocaine and methamphetamine addicts that have been linked to early onset of Parkinson's-like symptoms [3, 4, 10, 14]. Since its discovery, elucidations of physiological and pathological function of alpha-synuclein have been researched by a large number of laboratories. This review examines the existing literature pertaining to wild-type alpha-synuclein structure, its properties, its potential physiological and pathological functions, its interactions with other proteins such as dopamine transporter (DAT), and its role in modulating dopamine neurotransmission.

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Structure

Alpha-synuclein is a small 140 amino acid protein and a member of the synuclein family, which also includes beta and gamma-synuclein [19]. Alpha-synuclein is localized mainly at presynaptic terminals [19, 20] and has been shown to comprise up to ~1% of total proteins in the neuronal cytosol [21]. Alpha-synuclein consists of the amphipathic N-terminus involved in mediating lipid binding properties of the protein, the non-amyloid component (NAC) identified as the aggregation domain, and the acidic C-terminus, which is affiliated with calcium binding and inhibition of protein aggregation [19, 20] (Figure 1).

Despite its role in a number of pathological conditions coined synucleinopathies, which include Parkinson's disease and Dementia with Lewy Bodies and Multiple Systems Atrophy [4, 20, 22–24], the exact physiological function/s of alpha-synuclein is less understood. Recent reports suggest alpha-synuclein might be involved in regulation of normal cellular function via interaction with a number of binding proteins listed in Table 1, as well as neurotransmitter release, vesicular trafficking and oxidative stress [12, 25–29].

Alpha-synuclein is implicated in both sporadic and familial forms of PD. In addition to duplication and triplication of the gene that encodes alpha-synuclein (SNCA) [11, 30], N-terminal missense point mutations of alpha-synuclein, namely A53E, A53T, A30P, E46K, H50Q, and G51D, have shown a strong correlation with the autosomal dominant form of PD [31–34]. Alpha-synuclein undergoes a number of post-translational modifications including nitration [35], ubiquitination [36] and C-terminal truncation [37]. Alpha-synuclein inclusions are generally hyperphosphorylated. While Ser129 is the most commonly studied alpha-synuclein phosphorylation site [38, 39], Ser87 [40] and Tyr125 [41] are additional sites that can undergo phosphorylation and have been identified in Lewy bodies.

Animal Models of Wild-type Alpha-synuclein Knockout

To examine the physiological function of alpha-synuclein and its role in synuclein-dependent pathologies multiple lines of alpha-synuclein knockout and alpha-synuclein overexpressing animal models have been generated. In 2000, Abeliovich et al. generated an alpha-synuclein knockout mouse (alpha-Syn^{-/-}) via deletion of the first two exons, which encode amino acids 1–41 and upstream untranslated sequences [25]. These animals were shown to be viable, fertile and possess the capabilities to undergo standard uptake and release of dopamine (DA) in response to electrical stimulus. However, reminiscent to DAT KO animals [42–46], alpha-Syn^{-/-} mice exhibited a reduction in striatal dopamine content and inhibition of dopamine-dependent locomotor response to amphetamine, a psychostimulant known to increase locomotion via a DAT-dependent mechanism [47, 48]. These findings strongly support the hypothesis that alpha-synuclein is involved in regulation of dopamine neurotransmission [17, 18, 49, 50]. Alpha-synuclein regulation of dopaminergic neurons was further studied by Garcia-Reitboeck et al. in mice carrying a spontaneous deletion of the alpha-synuclein locus [51]. They found that deletion of the alpha-synuclein gene resulted in a decrease in the number of dopaminergic neurons in the substantia nigra further supporting the idea that physiological levels of alpha-synuclein may play a role in growth and development of neurons within the brain [51].

In contrast to animal models with deletion of alpha-synuclein, mice with triple synuclein knockout (TKO) (alpha, beta and gamma synuclein) generated by Burre et al. demonstrate age-dependent neurological impairments and decreased SNARE assembly, a cellular processes required for vesicular-mediated neurotransmitter release [15]. Knockdown of alpha, beta and gamma synucleins decreased the life expectancy of these animals [15]. Although initially there were no gross phenotypic differences in TKO alpha-synuclein mice as compared to wild type, aged TKO mice exhibited severe neurological impairments, and 50% of mice died at 500 days [15]. Disruption of SNARE assembly in cultured neurons from the TKO mice was rescued by replacement of exogenous alpha-synuclein [15]. These studies suggest beta and/or gamma synucleins may compensate for some of the physiological properties of alpha-synuclein.

A number of behavioral paradigms including the Morris water maze, rod suspension and open field tests have been used to determine whether alpha-synuclein deletion affects behavioral responses such as locomotion, impulsivity and cognition. For example, Kokhan et al. found complete deletion of the SNCA gene reduces learning working and spatial memory [52]. Pena-Oliver et al. reported a decrease in impulsivity as measured by the 5-choice serial reaction time task in C57BL/6J^{OlA}Hsd animals void of SNCA gene [53–55].

Animal Models of Wild-type Alpha-synuclein Overexpression

Developing animal models to recapitulate the pathophysiology of synucleinopathy and characteristic phenotypes of PD-like symptoms has been challenging. To date, more than a dozen alpha-synuclein overexpressing animal models (Table 2) as well as animal models containing alpha-synuclein point mutations have been generated to study synucleinopathy. Over the past two decades, generally two models of alpha-synuclein overexpression have been used: viral-mediated alpha-synuclein overexpression and transgenic animal models. Here, we focus only on transgenic animal models of full-length wild-type alpha-synuclein overexpression. In 2000, Masliah et al. generated a human alpha-synuclein overexpressing model driven by the PDGF- β promoter [56]. At 12 months, these mice had a decrease in tyrosine hydroxylase (TH) activity and levels within the striatum, decrease in TH positive fibers, and exhibited a significant decrease in dopamine levels compared to wild-type controls. Comparable disruptions in dopamine and TH levels and activity were observed in a GFP-tagged alpha-synuclein model generated by Hansen et al. [57]. These results strongly suggest that increased levels of alpha-synuclein result in a direct insult to the dopaminergic system. Additional alpha-synuclein mouse models were generated that displayed similar deficit in the dopaminergic system, but with some variability in the location of the pathology and alterations in phenotype. For example, neither model generated by Kahle et al. nor Matsuoka et al., driven by the mu-Thy-1 and rat TH promoter respectively, showed signs of motor deficits associated with PD [58, 59]. These findings triggered speculation that alpha-synuclein pathology alone might not be sufficient to cause full scale PD pathology. A comparative study by Rockenstein et al. [60] revealed significant differences between animal models driven by the PDGF- β promoter compared to that of the mu Thy-1 promoter. Among the differences, Rockenstein et al. found that alpha-synuclein overexpression driven by PDGF- β was moderate and was found in fewer brain regions as compared to alpha-synuclein expression driven by the mu Thy-1 promoter. This information further highlights the role of

promoter specificity in generating transgenic animal models and cloning strategies in general.

Disruptions in dopaminergic signaling have been documented in at least two recent animal models of alpha-synuclein overexpression. In a bacterial artificial chromosome (BAC) alpha-synuclein overexpressing mouse model generated by the Janezic's group, early stage decrease in dopamine release was measured via *in vivo* recording. The measured early stage attenuation of dopamine release in this animal model is attributed to decreased vesicle clustering that precedes the changes in the firing activity of dopamine neurons [61]. Importantly, these changes were only present in the nigrostriatal dopaminergic neurons [61]. Lam et al. generated an overexpressing alpha-synuclein mouse model driven by the Thy1 promoter [50]. These animals exhibit overexpression of alpha-synuclein in the brain regions implicated in PD. At 6 months old, these animals exhibited elevated dopamine levels in striatal tissue and increased open field activity but exhibited no change in dopamine uptake or amphetamine-induced dopamine efflux. However, at 14 months old these animals showed a profound decrease in dopamine and TH levels, indicative of age-dependent degeneration of dopaminergic neurons associated with pathological levels of alpha-synuclein. These findings suggest, prior to substantial neuronal loss and severe motor deficits, there are early, seemingly subtle, disruptions in dopamine neurotransmission. Identifying early stage pathologies and the cellular adaptations following alpha-synuclein elevation before neuronal demise may be the key for early diagnoses and development of effective therapies for the treatment of PD.

Similar observations have been made in the rat model of alpha-synuclein overexpression. Stoica et al. showed a decrease in dopamine levels in the striatum and accumulation of alpha-synuclein in multiple brain regions using a spontaneously inherited autosomal recessive rat model (Table 2) [62]. Importantly, none of the existing transgenic animal models of alpha-synuclein overexpression recapitulates *all* of the pathophysiological conditions associated with PD. This makes it increasingly difficult to pinpoint the specific cellular machinery within the dopamine system that is disrupted by alpha-synuclein overexpression. Over the last decade investigators have examined some of the prototypical cellular and molecular targets involved in neurodegeneration to understand how alpha-synuclein overexpression influences and disrupts neuronal activity before cell death. A few of these cellular responses are reviewed below.

Ca²⁺ Signaling

Ca²⁺ regulates multiple signaling mechanisms, neuronal health, and neurotoxicity. Dysregulation of intracellular Ca²⁺ signaling increases the susceptibility of the neuron to cell death [63–66]. Increased neuronal alpha-synuclein species, including oligomeric and aggregate forms of alpha-synuclein, have been proposed to elicit toxic effects on dopamine neurons and mammalian cell systems via disruption of Ca²⁺ homeostasis at rest and following electrical or pharmacological stimulation [67–69]. Although the exact role of pathological levels of alpha-synuclein on Ca²⁺ signaling remains unknown, a growing number of studies have explored the effects of alpha-synuclein on neuronal Ca²⁺ homeostasis. For example, Adamczyk et al. showed that application of 10 μM of wild-type

alpha-synuclein or the NAC domain of alpha-synuclein alone to rat synaptoneurosome preparations induced Ca^{2+} influx via N-type voltage-dependent Ca^{2+} channels [68]. Beta-synuclein, which lacks a NAC domain [70], had no effect on Ca^{2+} influx in this experimental paradigm. These results suggest while the NAC region is mainly associated with protein aggregation, it may also mediate Ca^{2+} influx. A recent study by Ronzitti et al. suggests that elevation of extracellular monomeric form of alpha-synuclein mediates Ca^{2+} increase via the Cav2.2 N-type Ca^{2+} channel in cortical neurons [71], confirming Adamczyk's report [68]. The alpha-synuclein regulation of Ca^{2+} channels in striatal slices has been shown to stimulate dopamine release, potentially via both classical neurotransmission mechanism and efflux of dopamine via DAT [18, 71]. Ronzitti et al. postulated that dysregulation of lipid rafts by alpha-synuclein overexpression causes a shift in Cav2.2 channels from cholesterol rich to cholesterol poor membrane microdomains thus altering Cav2.2 function as well as dopamine release. Although the report by Hettiarachchi et al. is not consistent with the above studies, it supports the general idea that abnormal alpha-synuclein levels increase intracellular Ca^{2+} levels in human neuroblastoma SH-SY5Y overexpressing wild-type alpha-synuclein [69] without any effect on the basal Ca^{2+} permeability. While only the Ronzitti's group experimentally identified the monomeric form of alpha-synuclein in their experiments, the studies by Adamczyk et al. and Hettiarachchi et al. did not identify the form of alpha-synuclein involved in increased neuronal Ca^{2+} influx. An elegant study by Danzer et al. demonstrated alpha-synuclein oligomers, but not monomers, increase the magnitude and duration of Ca^{2+} mobilization in SH-SY5Y cells and cortical neurons in response to high concentration (6 μM) of ionomycin [67]. Although these studies provide important insights about alpha-synuclein regulation of neuronal Ca^{2+} homeostasis, several caveats should be noted. First, the existing methodologies for the identification of alpha-synuclein oligomers have yet to address this highly contentious issue; secondly, unpredictable quantities of oligomers penetrate into the cells; and thirdly, the methods for the detection of the oligomers vs. monomers in these studies are derivative at best.

Recent work investigated the role of elevated alpha-synuclein on Ca^{2+} dynamics *in vivo*. Reznichenko et al. used two-photon microscopy to examine the spontaneous and stimulus-induced neuronal activity in the barrel cortex of transgenic mice expressing wild-type human alpha-synuclein (see Table 2), alpha-synuclein knockout mice, and wild-type controls [72]. This study revealed that following repetitive electrical stimulation (train of 3 stimuli at 3 Hz) alpha-synuclein overexpression altered both spontaneous and stimulus evoked Ca^{2+} responses, increased the spontaneous neuronal firing and Ca^{2+} entry per spike, and disrupted the buffering capacity of intracellular Ca^{2+} stores by increasing the decay time. Since ATP-dependent pumps and exchangers maintain the steep ionic gradient for Na^+ , Cl^- , K^+ , and Ca^{2+} across the plasma membrane, the disruption of neuronal Ca^{2+} homeostasis can lead to disruption of energy expenditure and therefore neuronal vulnerability, action potential dependent (classical neurotransmission) and action potential independent neurotransmitter release.

Neurotransmitter Release

Numerous studies have demonstrated that alpha-synuclein is localized at synaptic terminals; therefore, investigating the role of alpha-synuclein on synaptic transmission has provided critical information about the role of alpha-synuclein on cellular homeostasis prior to neurodegeneration.

Neurotransmitter release is a highly modulated event that is dependent on the function of SNARE protein complex assembly [73, 74]. Following early studies identifying the potential role of alpha-synuclein in synaptic neurotransmission, Burre et al. demonstrated that alpha-synuclein modulates neurotransmitter release via direct interaction with synaptobrevin-2, a major component of SNARE complex assembly [15]. The observation in triple synuclein knockout mice with a significant decrease in SNARE complex assembly further supports these findings [15]. Studies by Cooper et al. suggest alpha-synuclein may regulate ER-to-golgi trafficking of vesicles via a Rab1 dependent mechanism [26]. A follow up study by Gitler et al. also demonstrated that increased levels of alpha-synuclein decrease ER-to-golgi vesicular trafficking [75]. Abeliovich et al. have shown alpha-synuclein KO mice exhibited exaggerated levels of dopamine release following stimulation, suggesting a modulatory role for alpha-synuclein in dopamine neurotransmission [25]. In another study, Senior et al. reported alpha and gamma-synuclein null mice exhibited an increase in neurotransmitter release by increasing the release probability of synaptic vesicles [76]. In cultured hippocampal neurons, Murphy et al. have shown suppressed expression of alpha-synuclein in distal pools of synaptic vesicles [77]. A subsequent follow-up study did not reproduce these findings [78]. A later study by Larsen et al. found overexpression of alpha-synuclein reduced evoked release of neurotransmitters via inhibition of vesicular priming following the docking of vesicles to the membrane; while Mosharov et al. have shown alpha-synuclein overexpression increases cytosolic catecholamine concentration [79, 80]. Utilizing adeno-associated virus-type vector to overexpress alpha-synuclein in the substantia nigra of rats, Gaugler et al. showed an age-dependent decrease in dopamine release [81]. These findings suggest that the decrease in neurotransmitter release might be due to a decrease in dopamine containing vesicles and/or synaptic contact of these vesicles [81].

Surprisingly, few studies exist that have directly examined dopamine releases following alpha-synuclein overexpression. *In vivo* amperometry studies by Lunblad et al. revealed a decrease in dopamine release following KCl pulses and progressive axonal damage in 3-week-old triple synuclein knockout mice that amplified with age. These animals developed classical motor deficits seen in Parkinson's disease [82]. Although these studies are highly significant, they cannot isolate the action potential-dependent vs. action potential-independent dopamine release as they were performed in the absence of dopamine transporter blockade, known to mediate dopamine release via a reverse transport mechanism [48]. In addition to regulating dopamine neurotransmission, alpha-synuclein has also been implicated in glutamatergic neurotransmission. Diogenes et al. has found treating hippocampal slices with the oligomeric form of alpha-synuclein affects glutamatergic synaptic transmission in the CA1 region of hippocampus decreasing long-term potentiation [83].

Alpha-synuclein Regulation of Dopamine Synthesis, Storage, Clearance and Efflux

Alpha-synuclein Modulation of Tyrosine Hydroxylase

The influence of alpha-synuclein on the disruption of the dopamine system has been extensively studied. The clinical efficacy of levodopa, a dopamine precursor, suggests that one of the core symptoms of PD is related to alteration in dopamine neurotransmission. Although the literature supports the idea that decreases in the number of tyrosine hydroxylase (TH) positive neurons in the substantia nigra is associated with PD [84], it is important to note that a large number of reports have shown dysregulation of TH, DAT, and vesicular monoamine transporter function/levels/activity occurs prior to neuronal death. Increased wild-type alpha-synuclein level affects dopamine neurotransmission at multiple levels: synthesis, storage, recycling, reuptake and efflux of dopamine. Increased alpha-synuclein levels have been shown to decrease dopamine synthesis by decreasing the synthesis and activity of the rate-limiting enzyme TH via a direct interaction in dopamine neurons or cells expressing both proteins [85]. In addition, in MN9D cells alpha-synuclein overexpression has been shown to reduce TH activity by stabilizing TH in its inactive unphosphorylated form [86]. Alpha-synuclein decreases TH phosphorylation at Ser40 by inhibiting protein phosphatase 2A [86]. In addition, human and primate studies support an inverse relationship between increased levels of alpha-synuclein and TH expression [87], which further supports the idea that alpha-synuclein overexpression may affect dopamine production in the dopamine neuron.

Alpha-synuclein Modulation of VMAT2

Following its synthesis, dopamine is stored in the synaptic vesicles via vesicular monoamine transporter 2 (VMAT2), a function that is thought to reduce the harmful oxidative properties of dopamine metabolites [88]. The disruption of dopamine sequestration into the synaptic vesicles is thought to be one of the early events in degeneration of dopamine neurons [89–92]. Disruption in the expression or activity of VMAT2 has shown to increase oxidative stress [49]. Alpha-synuclein overexpression has been linked to modulation of VMAT2 levels and activity [93]. Supporting this idea, Ulusoy et al., found a decrease in vesicular dopamine storage in the nigral neurons followed by increased degeneration of dopamine neurons [94]. Consistently, knockdown of alpha-synuclein increased the amount of VMAT2 while overexpression of alpha-synuclein attenuated VMAT2 activity [94]. The idea that oxidation of un-sequestered cytosolic dopamine and its metabolites increase degeneration of dopaminergic neurons [95, 96] has led to the hypothesis that enhancing dopamine sequestration by VMAT2 may decrease alpha-synuclein toxicity. While this concept may hold true in the animal models of PD or *in vitro* models of synucleinopathy, more than half a century of clinical data have shown levodopa, which increases cytosolic and vesicular dopamine levels in patients with PD, does not decrease or accelerate the progression of the disease [97–100]. Therefore, alpha-synuclein mediated increase in cytosolic concentrations of dopamine alone may not be a significant source of alpha-synuclein-mediated neurotoxicity.

Alpha-synuclein modulation of Dopamine Transporter (DAT) Trafficking

Changes in constitutive DAT trafficking have been shown to regulate dopamine neurotransmission in the brain. A limited number of studies have explored whether increased levels of alpha-synuclein affect constitutive or regulated DAT trafficking. DAT trafficking has not been studied in alpha-synuclein knockout animals. The existing information about alpha-synuclein regulation of DAT trafficking is contradictory. For example, while Lee et al. demonstrated an increase in surface DAT levels following overexpression of alpha-synuclein in Ltk⁻ cells [101], Wersinger et al. showed a decrease in cell surface distribution of DAT when alpha-synuclein was overexpressed [102]. Similar findings were recapitulated by Oaks et al. in HEK293 and SK-N-MC cells showing sequestration of DAT in the ER-golgi pathway following alpha-synuclein overexpression [103]. In a follow up study, Wersinger et al. claimed trypsin rescues cell surface distribution of DAT in cells overexpressing wild-type alpha-synuclein [104]. Contrary to these studies, Swant et al. reported no change in membrane distribution DAT following alpha-synuclein overexpressing [17]. These conflicting results could be due to a number of reasons including but not limited to 1) the amount of alpha-synuclein in the cell, 2) alpha-synuclein conformation and 3) the model system used.

Alpha-synuclein modulation of Dopamine Clearance

Neurotransmitter uptake by transporter proteins is a major mechanism for terminating synaptic transmission. High affinity transport systems have been identified for the neurotransmitters dopamine, norepinephrine, serotonin, GABA, glutamate, and glycine [105–107]. DAT is a presynaptic membrane protein critical to dopamine homeostasis, as evidenced by transporter knockout studies, where long-term decrease in dopamine uptake (dopamine recycling) leads to hypo-dopamine state [42, 43, 108–110]. Similar to alpha-synuclein, DAT is implicated in neurological disorders such as Parkinson's disease [111, 112], schizophrenia [113, 114], psychostimulant abuse [115–118], and attention deficit hyperactivity (ADHD) [119–121]. The importance of DAT activity on synaptic dopamine levels was revealed in DAT-KO mice [42, 43, 118]. The DAT KO animals exhibit a profound increases in extracellular dopamine and significant depletion of intra-neuronal concentrations of dopamine [43, 118, 122]. Therefore, long-term decrease in DAT activity can lead to lower dopamine content, where the rate of synthesis determines the dopamine content.

It is well established that alpha-synuclein co-immunoprecipitates with DAT in transfected cells, rat primary mesencephalic dopaminergic neurons, and striatal tissue [18, 102, 123]; however, the consequence of this interaction on dopamine uptake via DAT is less understood. While Chadchankar's group reported a decrease in dopamine uptake in the dorsal striatum of alpha-synuclein knockout mice, *in vitro* and *in vivo* studies suggest increased alpha-synuclein levels decreased DAT-mediated dopamine uptake [17, 102]. *In vitro* studies by Wersinger et al., and Swant et al. have shown alpha-synuclein overexpression decreases dopamine uptake or uptake of a fluorescent substrate of DAT, respectively [17, 102]. Similarly, Pelkonen et al. have shown that microinjection of alpha-synuclein into the dorsal striatum of alpha-synuclein KO or wild-type mice decreases dopamine uptake six days after the microinjection without effecting acute dopamine uptake

[124]. These results contradict a report by Lee et al., where they have shown that alpha-synuclein increases dopamine uptake [123]. Since DAT operates in multiple modes (i.e., forward, reverse, and channel mode), an increase in dopamine efflux may affect the measured net dopamine uptake. Recently two different studies have shown that increased alpha-synuclein at or near the surface membrane enhances the interaction between DAT and alpha-synuclein, thus altering the ionic conductance of the transporter that can uniquely affect the excitability of dopaminergic neurons and thus dopamine neurotransmission [17, 18].

Alpha-synuclein modulation of DAT-mediated dopamine efflux

Dopamine can be released by two mechanisms: 1) vesicular release, the classic mechanism for neurotransmitter release, and 2) transporter-mediated dopamine efflux. These two mechanisms are differentially affected by inhibitors of DAT [125]. Like other neurotransmitters, dopamine is released from synaptic vesicles fused with the plasma membrane following an action potential [125–127]. Alpha-synuclein regulation of action potential dependent neurotransmitter release is described above. Efflux of dopamine, on the other hand, is mediated through DAT in an action potential independent process [42, 125, 128, 129]. The reverse transport of dopamine or dopamine efflux is critical for maintenance of tonic dopamine signaling and salient motivational states [130, 131]. Since, DAT-mediated dopamine efflux is regulated by intracellular Ca^{2+} [132] it is possible that alpha-synuclein induced increase in intracellular Ca^{2+} regulates the action potential independent release of dopamine via DAT. Consistent with this idea, Lam et al. have shown mice overexpressing alpha-synuclein exhibit an elevated extracellular dopamine concentration in the striatum that precedes dopamine loss in the striatum [50]. The increase in extracellular dopamine levels following alpha-synuclein overexpression might be due to decreased dopamine uptake, increased dopamine efflux, or combination of both mechanisms. Swant et al. have shown elevated alpha-synuclein decreases the initial slope of dopamine uptake and then within five minutes reaches the uptake level of cells not expressing alpha-synuclein [17]. Therefore, the alpha-synuclein mediated decrease in dopamine uptake alone, might not be the sole underlying mechanism for the elevated extracellular dopamine levels. In a recent study, Butler et al. have shown alpha-synuclein overexpression increases the DAT-mediated, nomifensine-sensitive basal dopamine efflux at resting membrane potential [18]. These reports are in agreement with the emerging consensus that alpha-synuclein regulates dopamine neurotransmission by influencing the action potential independent dopamine release via DAT.

Conclusions

Although multiplication of alpha-synuclein gene in human results in a seemingly modest increase in the amount of alpha-synuclein protein, this is still sufficient for the development of PD and/or dementia with lewy bodies [11]. While progressive decline in vulnerable substantia nigra pars compacta neurons are reported independently of overt protein aggregation, which supports a toxic role for non-aggregate (potentially monomeric) forms of alpha-synuclein [133–135], similar number of reports suggest that fibrillar alpha-synuclein inclusions damage dopamine neurons in the substantia nigra pars compacta [136]. This is

still a highly debated issue. What is clear, however, is that overexpression of wild-type alpha-synuclein decreases dopamine neurotransmission. Alpha-synuclein overexpression decreases TH and VMAT2 expression and activity, decreases DAT-mediated dopamine uptake and increases DAT-mediated dopamine efflux; thus, long-term alpha-synuclein overexpression decreases intracellular and extracellular levels of dopamine ultimately decreases dopaminergic neurotransmission in the brain.

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Figure 1.

Schematic representation of the wild-type full-length alpha-synuclein protein structure. N-terminal missense point mutations are found in the amphipathic and lipid-binding portion of the protein along with 7, 11mer repeats found throughout the N-terminus and Non-Amyloid Component (NAC) domain. The NAC region promotes aggregation while the acidic C-terminus inhibits aggregation of alpha-synuclein.

Table 1

Partner proteins of wild-type alpha-synuclein

Partner Protein	Overall Finding	Reference
14-3-3	Structural and functional homology to alpha-synuclein and has similar binding partners	[1]
Polo-like Kinase 2 (PLK2)	Mediates Ser129 phosphorylation on alpha-synuclein	[2]
PKC	Activity inhibited by elevated levels of alpha-synuclein	[1]
ERK	Activity inhibited by elevated levels of alpha-synuclein	[5]
Phospholipase D2 (PLD2)	Decreased activity by Alpha-synuclein	[4, 6]
PKA	Stimulates activity resulting in phosphorylation of tau mediated by alpha-synuclein	[8]
Synphilin-1	May act as an adaptor protein for anchoring of alpha-synuclein to other proteins	[9]
Poly Unsaturated Fatty Acids (PUFA)	Upon binding to alpha-synuclein, exhibits significant reduction in membrane fluidity	[12]
BDNF	Decrease in expression due to inhibition of required transcription factors mediated by alpha-synuclein	[13]
Synaptobrevin	Binding to SNARE complexes inhibited by alpha-synuclein overexpression	[15]
Focal Adhesion kinase pp125	Phosphorylates Tyr136 on alpha-synuclein to improve behavioral performance	[16]
Dopamine transporter (DAT)	Increased DAT-mediated inward current leading to membrane depolarization, increased dopamine efflux, and decreased dopamine uptake	[17, 18]

Table 2

Alpha-synuclein overexpressing mouse models (mutant models and viral-mediated alterations in alpha-synuclein overexpression are not included)

Promoter	DA system disturbances	Brain Region expression	Phenotype	Reference
PDGF- β	Decrease in TH activity, levels and fibers; decrease in DA levels.	Neocortex, olfactory bulb and midbrain and glial expression	Motor deficits displayed at 12 months	[42]
Mu Thy-1	N/A	Substantia nigra, striatum, hippocampus, cortex and brainstem	N/A	[44]
Rat TH	N/A	Striatum, cerebellum, and olfactory bulb	N/A	[125]
Mouse prion	N/A	Spinal cord, brainstem, cerebellum, thalamus, and striatum	Middle age onset, hunched back freezing paralysis	[126]
Rat TH	Decrease in DAT density and decrease in DA and metabolite levels	Substantia nigra, locus ceruleus, ventral tegmental area, and striatum	Middle age onset. Decrease in locomotor activity and coordination	[127]
Proteolipid	N/A	Cerebellum and mature oligodendrocytes	N/A	[128]
mu Thy-1	N/A	Basal ganglia, thalamus and substantia nigra	N/A	[46]
Mouse prion	N/A	Olfactory bulb, cortex, striatum, forebrain, hippocampus, midbrain, spinal cord and cerebellum	Decrease in stride length, grip strength, vertical activity and rotarod	[129]
Thy-1	Elevated striatal DA levels at 6 months; at 14 months, decreased TH expression, DA levels in the striatum and abnormal DA modulation of spontaneous EPSCs	Nigrostriatal pathway	Sensory motor deficits at 14 months	[49]
Spontaneous autosomal recessive model	Decrease of DA levels in the striatum. Decrease in TH levels in the striatum	Substantia nigra, brainstem, and striatum	15 days postnatal, rats exhibit tremor, rigidity, spasticity and postural instability	[50]
Mouse alpha-synuclein promoter	Reduction in striatal DA release	Substantia nigra, ventral tegmental area, olfactory bulb and neocortex	Reduction in AMPH locomotor response, abulatory movement at 7 months and latency to fall at 12 months	[43]
Bacterial artificial chromosome.	Early stage decrease in DA release followed by severe loss of dopaminergic neurons at later stages	Caudate putamen, nucleus accumbens, and ventral tegmental area	Reduced latency to fall at 18 months with reduced forepaw stride and poor performance on rotarod	[48]