TrkB neurotrophic activities are blocked by α-synuclein, triggering dopaminergic cell death in Parkinson's disease

Seong Su Kang[a], Zhentao Zhang[a,b], Xia Liu[a], Fredric P. Manfredsson[c], Matthew J. Benskey[c], Xuebing Cao[d], Jun Xu[e,1], Yi E. Sun[f,g], and Keqiang Ye[a,f,g,1]

[a]Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, GA 30322; [b]Department of Neurology, Renmin Hospital of Wuhan University, Wuhan 430060, China; [c]Translational Science and Molecular Medicine, College of Human Medicine, Michigan State University, Grand Rapids, MI 49503; [d]Department of Neurology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China; [e]East Hospital, Tongji University School of Medicine, Shanghai 200120, China; [f]Translational Center for Stem Cell Research, Tongji Hospital, Tongji University School of Medicine, Shanghai 200065, China; and [g]Department of Regenerative Medicine, Tongji University School of Medicine, Shanghai 200065, China

Edited by Solomon H. Snyder, Johns Hopkins University School of Medicine, Baltimore, MD, and approved August 24, 2017 (received for review August 7, 2017)

BDNF/TrkB neurotrophic signaling is essential for dopaminergic neuronal survival, and the activities are reduced in the substantial nigra (SN) of Parkinson's disease (PD). However, whether α-Syn (alpha-synuclein) aggregation, a hallmark in the remaining SN neurons in PD, accounts for the neurotrophic inhibition remains elusive. Here we show that α-Syn selectively interacts with TrkB receptors and inhibits BDNF/TrkB signaling, leading to dopaminergic neuronal death. α-Syn binds to the kinase domain on TrkB, which is negatively regulated by BDNF or Fyn tyrosine kinase. Interestingly, α-Syn represses TrkB lipid raft distribution, decreases its internalization, and reduces its axonal trafficking. Moreover, α-Syn also reduces TrkB protein levels via up-regulation of TrkB ubiquitination. Remarkably, dopamine's metabolite 3,4-Dihydroxyphenylacetaldehyde (DOPAL) stimulates the interaction between α-Syn and TrkB. Accordingly, MAO-B inhibitor rasagiline disrupts α-Syn/TrkB complex and rescues TrkB neurotrophic signaling, preventing α-Syn–induced dopaminergic neuronal death and restoring motor functions. Hence, our findings demonstrate a noble pathological role of α-Syn in antagonizing neurotrophic signaling, providing a molecular mechanism that accounts for its neurotoxicity in PD.

neurodegenerative diseases | dopamine | Lewy bodies | substantia nigra

Parkinson's disease (PD) is the second most common neurodegenerative disorder, with typical movement abnormalities that include resting tremor, rigidity, bradykinesia, and postural instability as major clinical manifestation. PD is characterized by selective degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and a corresponding decrease in dopaminergic innervation of the striatum, along with the accumulation of intraneuronal protein aggregates [Lewy bodies (LBs) and Lewy neurites (LNs)] primarily composed of the protein alpha-Synuclein (α-Syn) (1, 2). The physiological functions of α-Syn remain unclear, but several studies have demonstrated that it might be implicated in dopamine (DA) biosynthesis, synaptic plasticity, and vesicular dynamics (3, 4). A pathogenic role for α-Syn in PD is supported by various genetic data. For instance, multiplications of the gene encoding α-Syn (*SNCA*) and various point mutations in this gene (e.g., A53T, A30P, and E46K) result in dominant familial parkinsonism (1, 5, 6). Moreover, certain polymorphisms in *SNCA* are major risk factors for sporadic PD (7). The abnormal accumulation of α-Syn, resulting from an unbalanced production and/or degradation of the protein, is thought to trigger DAergic neuronal death in both familial and sporadic cases of PD (8). So far, several cellular mechanisms, such as aberrant protein folding, oxidative stress, and mitochondrial dysfunction have been linked to the development and progression of PD, however the exact molecular mechanisms of how α-Syn exerts neurotoxicity remain elusive.

Neurotrophins (NTs) are growth factors that regulate the development and maintenance of the peripheral and the central nervous systems. Brain-derived neurotrophic factor (BDNF) is a member of the NT family, which includes nerve growth factor (NGF), NT-3, and NT-4/5. BDNF, like the other NTs, exerts its biological functions on neurons through two transmembrane receptors: the p75 neurotrophin receptor (p75NTR) and the TrkB receptor tyrosine kinase (NGF binds to TrkA, BDNF and NT-4/5 bind to TrkB, and NT-3 preferentially binds to TrkC) (9). TrkB is one of the most widely distributed neurotrophic receptors (NTRs) in the brain and is highly enriched in the neocortex, hippocampus, striatum, and brainstem. BDNF binding to TrkB triggers its dimerization through conformational changes and autophosphorylation of tyrosine residues in its intracellular domain (ICD), resulting in activation of the three major signaling pathways involving mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K), and phospholipase C-g1 (PLC-γ1), mediating neural differentiation, survival, and neurogenesis. BDNF colocalizes with DAergic neurons in the SN (10), and promotes DAergic neuronal survival in vivo (11). PD patients show markedly decreased levels of BDNF (12–14), indicating that reduced levels of this neurotrophic factor may be involved in the etiology and pathogenesis of PD. Transplantation of modified fibroblasts that express BDNF into either the striatum or the midbrain attenuates 6-hydroxydopamine (6-OHDA) or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced nigrostriatal

Significance

Alpha-synuclein plays an important role in the pathophysiology of Parkinson's disease (PD), however the molecular mechanisms related to α-synuclein in neurodegeneration of PD remain unknown. We show that α-synuclein specifically inhibits BDNF/TrkB signaling, leading to dopaminergic neuronal death. The disruption of this interaction rescues TrkB signaling, preventing α-Syn–induced dopaminergic neuronal death and restoring motor functions. This study reveals the mechanism related to α-synuclein–induced neurotoxicity of PD via regulation of TrkB neurotrophic signaling.

Author contributions: S.S.K., Z.Z., J.X., and K.Y. designed research; S.S.K., Z.Z., and X.L. performed research; F.P.M., M.J.B., X.C., and Y.E.S. contributed new reagents/analytic tools; S.S.K., J.X., and K.Y. analyzed data; and J.X. and K.Y. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

[1]To whom correspondence may be addressed. Email: xunymc2000@yahoo.com or kye@emory.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1713969114/-/DCSupplemental.

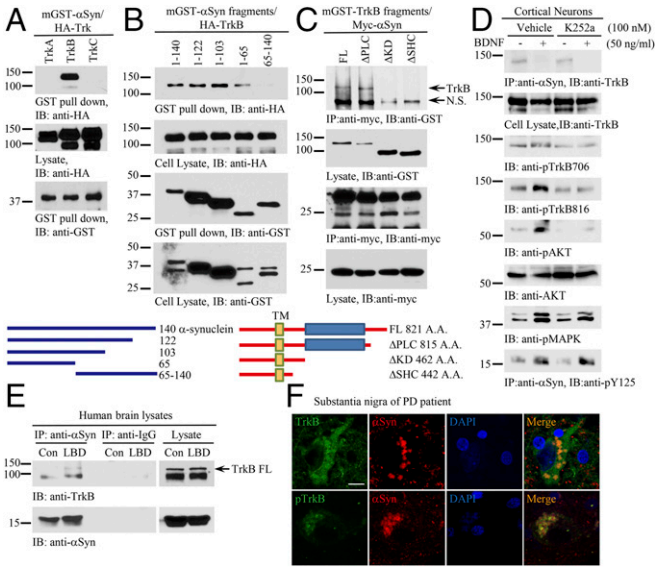

Fig. 1. α-Synuclein selectively interacts with TrkB receptors. (*A*) α-Syn specifically interacts with TrkB receptors. GST pull-down assay was conducted from HEK293 cells cotransfected with mammalian GST–α-Syn and HA-Trks. (*B*) α-Syn N terminus is implicated in binding TrkB. Different mGST-tagged α-Syn truncated were cotransfected with HA-TrkB into HEK293 cells. A GST pull-down assay was performed, and coprecipitated proteins were analyzed by immunoblotting with anti-HA (*Top*). Schematic diagram of α-Syn truncations (*Bottom*). (*C*) TrkB kinase domain is indispensable for α-Syn to interact with TrkB. (*Top*) Mapping assay for TrkB ICD required for binding to α-Syn. (*Bottom*) Schematic diagram of TrkB domains. (*D*) BDNF inhibits α-Syn/TrkB association. Cortical neurons were pretreated with K252a (100 nM) for 15 min, followed by BDNF treatment (50 ng/mL) for 30 min. Coimmunoprecipitation was performed with anti–α-Syn, and the coprecipitated proteins were analyzed by immunoblotting with anti-TrkB (*Top*). Cell lysates were probed with various antibodies (second through bottom panels). (*E*) α-Syn associates with TrkB in LBD human patient brains. Brain lysates from LBD patients were immunoprecipitated with control IgG or anti–α-Syn, and the coprecipitated proteins were analyzed by immunoblotting with anti-TrkB. (*F*) TrkB colocalizes with α-Syn in the LBs of PD patients. Immunofluorescent costainings with anti-TrkB or p-TrkB 706 (Green) and α-Syn (Red) were conducted with human PD brain sections. The nuclei were stained with DAPI. (Scale bar, 20 μm.)

degeneration (15). Also, BDNF can modulate dopaminergic neurotransmission in nigrostriatal neurons, as shown by elevated rotational behavior and increased turnover of DA in the striatum (16). BDNF can promote functional recovery from 6-OHDA lesions following adeno-associated virus (AAV)-mediated BDNF overexpression within striatal medium spiny neurons (17). Together, these observations strongly support an essential role of BDNF signaling for the survival of DAergic neurons, which when lost may contribute to the pathology observed in PD.

In this study, we report that α-Syn directly binds to the kinase domain on TrkB receptors and blocks BDNF/TrkB neurotrophic signaling pathways by suppressing the lipid raft distribution of the BDNF/TrkB complex, as well as the endocytosis and trafficking of the TrkB receptors, ultimately leading to DAergic neurodegeneration. Notably, α-Syn overexpression elicits a prominent reduction in TrkB protein levels, which is mediated by an increase in TrkB ubiquitination. Disruption of α-Syn/TrkB complex formation with the monoamine oxidase B (MAO-B) inhibitor rasagiline rescues BDNF signaling and prevents α-Syn–induced DAergic neuronal loss and the corresponding motor impairment. Hence, this innovative finding provides insight into a pathological role(s) of α-Syn in mediating BDNF/TrkB signal transduction and may represent an unappreciated mechanism by which a-Syn contributes to PD pathogenesis.

Results

α-Synuclein Interacts with TrkB. Due to the well-documented correlation between aberrant α-Syn accumulation and nigrostriatal degeneration, the association between reduced BDNF signaling and nigrostriatal degeneration, and the numerous studies revealing the cross-talk between α-Syn and BDNF (18–20), we hypothesized that pathological α-Syn might directly impinge on BDNF/TrkB pathway in PD. To test this hypothesis, we conducted a coimmunoprecipitation assay and found that α-Syn selectively interacted with the TrkB, but not TrkA or TrkC, receptor in cotransfected HEK293 cells (Fig. 1*A*). A truncation assay revealed that the N terminus of α-Syn was essential for binding to TrkB (Fig. 1*B*). Further, mapping studies demonstrated that the TrkB kinase domain on the intracellular motif was necessary for α-Syn binding to the TrkB receptor (Fig. 1*C*). To explore whether the kinase activity is necessary for mediating

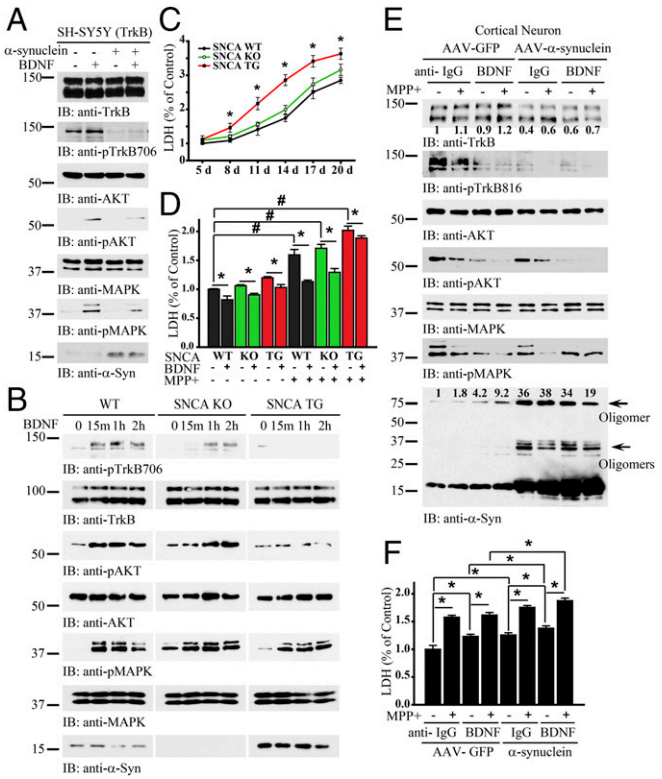

Fig. 2. Overexpression of α-Syn blocks BDNF/TrkB signaling. (*A*) α-Syn inhibits BDNF/TrkB signaling. TrkB stably transfected SH-SY5Y (BR6) cells were transfected with α-Syn, followed by treatment with BDNF for 10 min. p-TrkB and its downstream effectors, p-Akt and p-MAPK, were analyzed in the cell lysates. (*B*) BDNF/TrkB signaling is blocked in *SNCA* overexpressing transgenic neurons. Wild-type, *SNCA* KO, and *SNCA* transgenic neurons were treated with BDNF for 15 min, 1 h, or 2 h, and the pTrkB signaling cascade was probed with various antibodies. (*C*) LDH assay of *SNCA* transgenic, *SNCA* KO, and wild-type dopaminergic neurons. (*D*) α-Syn overexpression decreases the neuroprotective effects of BDNF against MPP+-induced neuronal cell death. MPP^+ sensitized α-Syn–induced neuronal cell death. Shown are *SNCA* transgenic, *SNCA* KO, or wild-type dopaminergic neurons in the presence or absence of BDNF, treated with MPP^+ (200 μM) or not for 24 h. LDH assay was conducted with cell medium. (*E* and *F*) Overexpression of α-Syn decreases TrkB levels and elevates neuronal cell death. Primary cortical cultures were infected with AAV virus expressing α-Syn or GFP control, followed by treatment with anti-BDNF or anti-IgG, then treated with MPP^+ (200 μM) for 24 h. Immunoblotting analysis of cell lysates with various antibodies (*E*) and LDH assay of the treated cells (*F*). Data are shown as mean + SEM. $n = 3$ each group. $^*P < 0.05$, $^{\#}P < 0.01$. The relative intensities of the band that were quantified with Image J were indicated in the immunoblots.

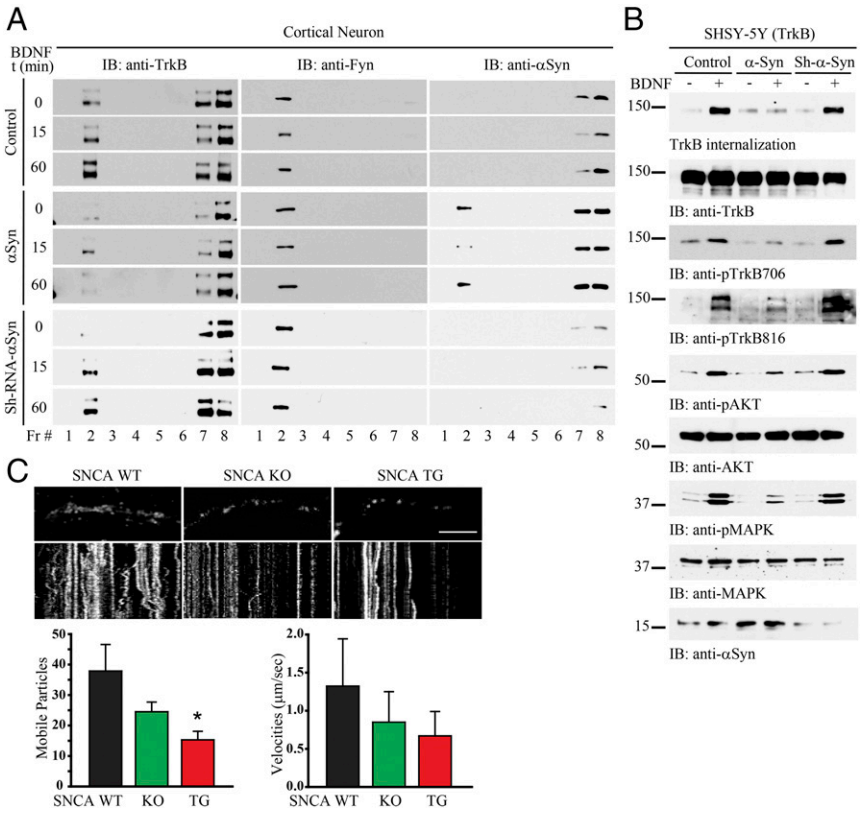

Fig. 3. α-Syn decreases TrkB lipid raft distribution, internalization, and axonal transportation. (*A*) Lipid raft distribution assay. Primary cortical cultures were infected with various AAV constructs overexpressing α-Syn or depleting α-Syn, followed by treatment with BDNF for 0, 15, and 60 min, respectively. The samples were subsequently subjected to subcellular fractionation. TrkB was dispensed in fraction #2, #7, and #8. Fyn, a well-characterized lipid raft resident, was distributed in fraction #2, with TrkB. (*B*) α-Syn decreases TrkB internalization and its downstream signaling. BR6 cells were transduced with the indicated virus, followed by treatment with BDNF for 15 min. TrkB internalization was conducted and monitored by immunoblotting. (*C*) Live-cell imaging. Different genotypes of dopaminergic neurons were transfected with GFP-TrkB and imaged 7 d later. Images were captured every 1 s for 3 min. Neurons were treated with BDNF for 30 min before imaging, and BDNF was included in the imaging media. Images are from movies captured every 1 s for 3 min. (Scale bar, 10 μm.) Kymographs shown below were generated as visual representations of distance traveled over time. Of the whole particles, the percentages of anterograde and retrograde mobile particles were quantified (*Left*). The speed of mobile TrkB-GFP particles was measured in BDNF-treated neurons (*Right*). Data are shown as mean + SEM. $n = 6–9$ axons each group. $^*P < 0.05$.

the interaction between TrkB and α-Syn, we pretreated rat primary cortical neurons with the Trk receptor inhibitor K252a, followed by BDNF treatment. Remarkably, BDNF completely disrupted the formation of the α-Syn/TrkB complex, and inhibition of TrkB barely affected the interactions (Fig. 1*D*, *Top*). As expected, BDNF-stimulated phosphorylation of TrkB (p-TrkB), and its downstream p-Akt and p-MAPK signals, was strongly blunted by K252a. Notably, BDNF strongly elicited α-Syn Y125 phosphorylation regardless of the treatment with K252a (Fig. 1*D*, *Bottom*). Interestingly, we found that α-Syn also bound to TrkB in human cortex samples from brains of patients with Lewy body dementia (LBD) but not in control brain tissue samples (Fig. 1*E*). Immunofluorescent staining also verified that TrkB colocalized with α-Syn in LBs in PD patients with p-TrkB signals much dimmer than total TrkB levels (Fig. 1*F*). Hence, α-Syn interacts with TrkB, which can be inhibited by BDNF treatment.

α-Synuclein Inhibits BDNF-Mediated TrkB Signaling. To examine the biological effect of α-Syn on BDNF/TrkB signaling, we transfected BR6 cells (SH-SY5Y cells stably transfected with the TrkB receptor) with α-Syn, followed by BDNF stimulation. BDNF-induced phosphorylation of TrkB and its downstream effectors was blocked by α-Syn overexpression (Fig. 2*A*). We also extended the experiment into primary cortical neurons from *SNCA* transgenic overexpressing mice or *SNCA* KO mice. In wild-type neurons, BDNF elicited prominent p-TrkB/p-Akt/p-MAPK signaling, which was significantly suppressed in *SNCA* transgenic neurons. In *SNCA* KO neurons, the BDNF-triggered signaling cascade remained intact, though the temporal onset of TrkB activation was delayed (Fig. 2*B*). One of the key physiological functions of the BDNF/TrkB pathway is to promote neuronal survival. Consequently, we performed a lactate dehydrogenase (LDH) release cytotoxicity assay to quantify spontaneous cell death. The LDH assay demonstrated that *SNCA* transgenic neurons exhibited much higher spontaneous cell death compared with wild-type or *SNCA* KO neurons (Fig. 2*C*). Treatment of primary neurons with BDNF significantly repressed neuronal cell death regardless of genotype (Fig. 2*D*). To investigate how α-Syn affects BDNF-mediated neuroprotection in the face of PD-associated pathology, we next treated primary neurons with the neurotoxicant 1-methyl-4-phenylpyridinium (MPP^+) and analyzed LDH release. MPP+-induced LDH release was strongly reduced in both wild-type and *SNCA* KO neurons by BDNF treatment. However, the neuroprotective effect of BDNF was much weaker in *SNCA* transgenic neurons (Fig. 2*D*). To further investigate the biological role of α-Syn in BDNF-mediated neuronal survival, we transduced primary cortical neurons with AAV-expressing human α-Syn. And neurons underwent BDNF deprivation (by treatment with an anti-BDNF antibody) either in the presence or absence of MPP^+. Within neurons transduced with the AAV-GFP control vector, depletion of BDNF diminished p-TrkB. MPP^+ also reduced p-TrkB, and the combination of both BDNF depletion and MPP+ treatment resulted in an additive effect to further decrease p-TrkB (Fig. 2*E*, second panel, left four lanes). Strikingly, AAV-mediated overexpression of α-Syn strongly repressed BDNF-induced phosphorylation of TrkB. The combination of α-Syn overexpression with MPP+ treatment completely abolished p-TrkB levels, and importantly this effect was independent of the presence or absence of BDNF. Moreover, total TrkB levels were reduced following α-Syn overexpression (Fig. 2*E*, top two panels). The downstream phosphorylation of Akt followed the p-TrkB pattern. P-MAPK displayed similar effects, except that p-MAPK 42 was not further diminished in the AAV–α-Syn-infected neurons treated with anti-BDNF (Fig. 2*E*, panels 3–6). Immunoblotting of α-Syn showed increased α-Syn, which was prominently displayed as oligomers in the presence of MPP^+ and anti-BDNF. Overexpression of α-Syn induced notable oligomerization (Fig. 2*E*, *Bottom*). We next analyzed cytotoxicity within these treatment groups. The LDH assay revealed an inverse correlation between levels of p-TrkB/p-Akt and LDH release. Neurons transduced with AAV–α-Syn exhibited more cell death

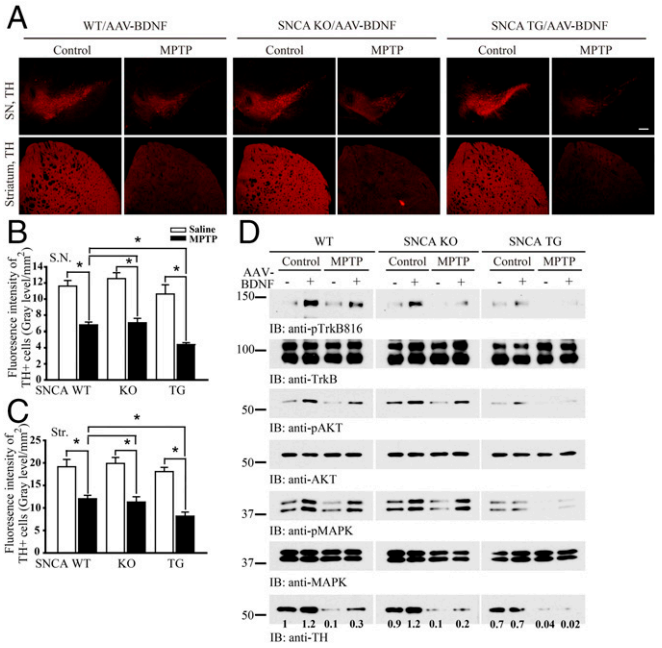

Fig. 4. α-Syn overexpression accelerates dopaminergic neuronal loss following MPTP administration. (*A*) α-Syn overexpression sensitizes TH neuronal loss induced by MPTP. *SNCA* transgenic, *SNCA* KO, or wild-type mice were injected with AAV-BDNF virus. After 2 wk, MPTP (30 mg·kg·d) was injected for 5 d. TH immunoreactivity within the SNpc and striatum was analyzed by immunofluorescent staining. (Scale bar, 200 μm.) (*B* and *C*) Quantification of TH-positive fluorescent signaling in SNpc (*B*) and striatum (Str) (*C*). Overexpression of α-Syn exacerbated MPTP-induced dopaminergic neuronal loss, which was reduced by BDNF. Data are shown as mean + SEM. $n = 6$ sections each group. $^*P < 0.05$. (*D*) Immunoblotting analysis of SNpc tissue lysates from the above animals. The relative intensities of TH that were quantified with Image J were indicated under the immunoblots.

than those transduced with AAV-GFP. Further, BDNF deprivation also significantly increased cell death. Finally, maximal cytotoxicity was observed following the combination of MPP^+ and BDNF deprivation (Fig. 2*F*). These data indicate that overexpression of α-Syn reduces TrkB protein levels as well as p-TrkB signaling, thereby inhibiting the neurotrophic effects of BDNF, resulting in much more robust neuronal death in the face of PD-associated toxicity.

α-Synuclein Blocks TrkB Signaling by Inhibiting Its Internalization and Lipid Raft Distribution. TrkB receptors reside within lipid rafts, and this localization is mediated by Fyn tyrosine kinase. Disrupting lipid rafts prevents the full activation of TrkB (21). To test whether α-Syn mediates TrkB cellular localization, we conducted a subcellular fractionation experiment. We prepared primary cortical neuronal cultures and transduced the cells with AAV overexpressing α-Syn or a short-hairpin RNA (shRNA) targeting endogenous α-Syn, followed by BDNF treatment for 0, 15, or 60 min. In control neurons, BDNF treatment resulted in a TrkB enrichment in fraction #2, where Fyn also eluted (i.e., this fraction also contained lipid rafts, as Fyn is also used as a marker for these; Fig. 3*A*). In addition, TrkB and α-Syn coeluted in fraction #7 and #8. When α-Syn was overexpressed, it distributed into fraction #2 as well. Notably, TrkB levels in lipid raft fractions were clearly reduced following α-Syn overexpression, whereas Fyn levels remained intact. Following α-Syn knockdown, TrkB levels in fraction #2 were increased upon BDNF stimulation, similar to control cells (Fig. 3*A*). The NT–Trk complex internalization is essential for the signal transduction that initiates the cellular response to target-derived NTs, and this process is regulated through clathrin-mediated endocytosis, leading to the formation of signaling endosomes (22, 23). Next, we examined whether α-Syn also regulates TrkB internalization in BR6 cells. BDNF strongly stimulated TrkB internalization in control cells and α-Syn shRNA-treated cells, associated with robust p-TrkB, p-Akt, and p-MAPK signals. Nonetheless, these effects were substantially antagonized by α-Syn overexpression (Fig. 3*B*). To further assess the effect of α-Syn on TrkB endocytosis, we performed immunofluorescent staining to monitor the subcellular localization of TrkB as well as the colocalization of TrkB with EEA1, a marker for early endosomes. BDNF induced the internalization of both TrkB and p-TrkB as indicated by colocalization with EEA1. These effects were reduced in *SNCA* transgenic neurons (Fig. S1). The trafficking of NT/Trk complexes within the endosome plays a critical role in neurotrophic signaling cascade. Accordingly, we performed a live-cell imaging assay using wild-type, *SNCA* transgenic, and *SNCA* KO dopaminergic neurons. We found that the mobility of TrkB fluorescent particles within the axon of *SNCA* overexpressing transgenic neurons was significantly impaired compared with that observed in wild-type or *SNCA* KO cells (Fig. 3*C*), fitting with previous reports that formation of α-Syn LN-like aggregates in axons impedes the transport of distinct endosomes (24).

α-Synuclein Blocks the Prosurvival Effects of BDNF/TrkB in Dopaminergic Neurons. To explore the biologic effect of α-Syn–mediated blocking of BDNF/TrkB neurotrophic activities in DAergic neuronal survival, we stereotaxically delivered AAV-GFP into the left SNpc and AAV-BDNF into the right SNpc of WT *SNCA* KO or *SNCA* transgenic overexpressing mice. Mice then received a daily i.p. injection of MPTP (30 mg/kg) treatment for 5 d. Immunofluorescent staining demonstrated that GFP and BDNF were appropriately expressed in the respective SNpc regions (Fig. S2*A*). BDNF– expression induced the p-TrkB/p-Akt/p-MAPK signaling cascade in WT and *SNCA* KO mice, however this effect was suppressed in *SNCA* transgenic mice (Fig. S2*B*). As expected, MPTP administration induced substantial DAergic neurodegeneration in all of the genotypes, but tyrosine hydroxylase (TH)-positive cell loss was significantly greater in *SNCA* transgenic mice compared with WT or *SNCA* KO mice (Fig. 4 *A*–*C*). Immunoblotting analysis demonstrated that TH immunoreactivity was reduced in *SNCA* transgenic mice compared with wild-type or *SNCA* KO mice, and MPTP treatment almost completely eliminated TH immunoreactivity in *SNCA* transgenic mice (Fig. 4*D*). Hence, α-Syn inhibits TrkB signaling, rendering DAergic neurons more vulnerable to the neurotoxin MPTP.

Disruption of α-Syn/TrkB Association Promotes Dopaminergic Neuronal Survival. The DA metabolite 3,4-dihydroxyphenylacetaldehyde (DOPAL) induces α-Syn binding to TrkB, leading to suppression of TrkB neurotrophic signaling and escalation of DAergic neurodegeneration (Fig. S3). Next, we tested whether the inhibition of DOPAL by MAO-B inhibitor (rasagiline) could block α-Syn/TrkB association, promoting DAergic neuron survival in vivo as well as in BR6 cells (Fig. S4). We injected mice with AAV-GFP or AAV–α-Syn into the left and right substantial nigra (SN) of the same mice, respectively, followed by rasagiline administration. As expected, overexpression of α-Syn elicited DAergic neurodegeneration in the SN and nigrostriatal denervation of the striatum compared with the expressing hemisphere. This effect was alleviated by rasagiline treatment (Fig. 5 *A*–*C*). Consequently, DA concentrations were significantly elevated in both SN and striatum (Fig. 5 *D* and *E*). Since MAO-B was potently inhibited by rasagiline, its oxidation product DOPAC was substantially reduced (Fig. 5 *F* and *G*). Motor behavioral assays indicated that α-Syn–induced motor dysfunctions were rescued by rasagiline (Fig. 5 *H*–*J*). Moreover, coimmunoprecipitation assays showed that rasagiline strongly inhibited the association between α-Syn and TrkB, restoring BDNF/TrkB neurotrophic signaling (Fig. 5*K*).

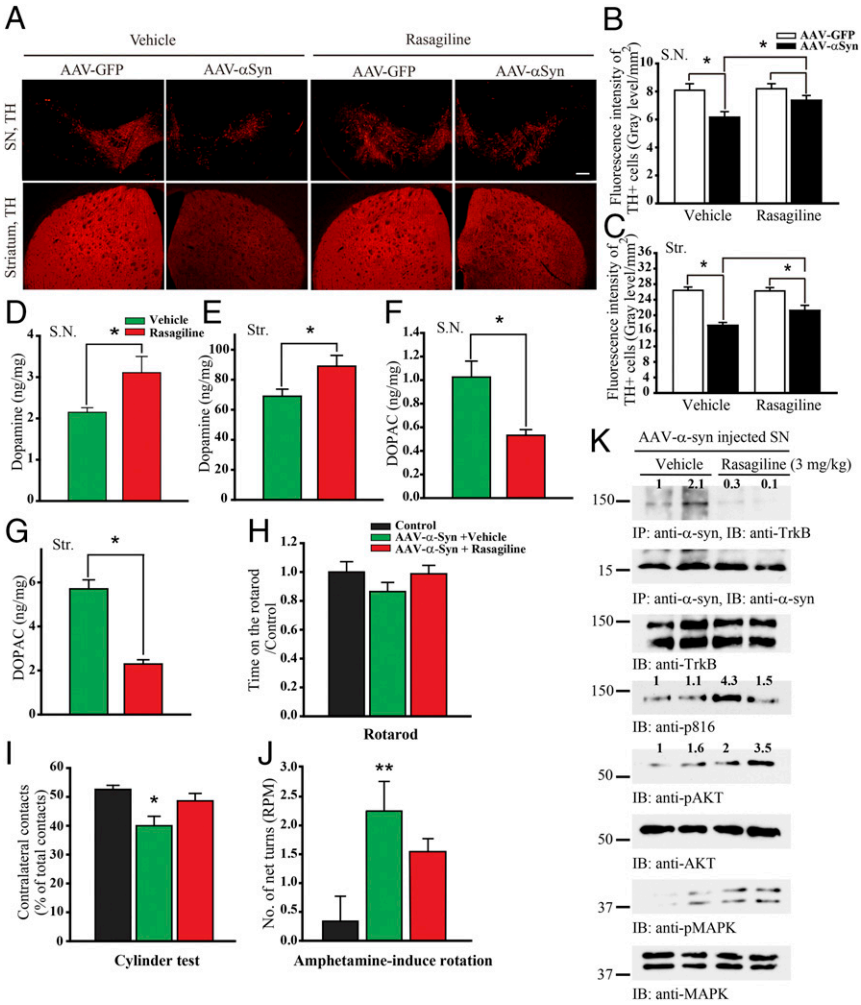

Fig. 5. Rasagiline disrupts α-Syn/TrkB complex and rescues dopaminergic neurons from α-Syn–induced cell death. (*A*) Rasagiline reduces TH loss induced by α-Syn. C57BL/6 mice were injected with AAV-GFP or AAV–α-Syn into the left and right SN, respectively, followed by rasagiline (3 mg·kg·d) treatment for 10 d. TH expression in SN and striatum was analyzed by immunofluorescent staining. (Scale bar, 200 μm.) (*B* and *C*) Quantification of TH-positive fluorescent signaling in SN (*B*) and striatum (Str) (*C*). Data are shown as mean + SEM. $n = 6$ sections each group. $^*P < 0.05$. (*D* and *E*) DA concentrations in SN and striatum were increased by rasagiline in α-Syn–overexpressed mice. (*F* and *G*) DA metabolite DOPAC concentrations in SN and striatum were decreased by MAO-B inhibitor, rasagiline, in α-Syn–overexpressed mice. Data are shown as mean + SEM. $n = 3$ each group. $^*P < 0.05$. (*H–J*) Motor behavioral assays. Overexpression of α-Syn induced motor impairment, and rasagiline significantly improved the motor functions. Data are shown as mean + SEM. $n = 8$ each group. $^*P < 0.05$, $^{**}P < 0.01$. (*K*) Rasagiline disrupts α-Syn/TrkB complex and restores p-TrkB signaling. Coimmunoprecipitation assay with anti–α-Syn from SN tissues treated with or without rasagiline and coprecipitated proteins were analyzed by immunoblotting. SN lysates were probed by various indicated antibodies.

Conceivably, rasagiline disrupts α-Syn/TrkB complex via inhibiting MAO-B–produced DOPAL.

Discussion

In the current study, we provide compelling evidence that α-Syn directly interacts with the TrkB receptors. This interaction is in turn negatively regulated by BDNF and Fyn tyrosine kinase activity, resulting in the phosphorylation of α-Syn on Y125, causing it to dissociate from TrkB receptors (Fig. S5). Strikingly, α-Syn binds TrkB and completely suppresses its neurotrophic activities, increasing the vulnerability of DA neurons to degeneration. Moreover, α-Syn potently reduces TrkB protein levels by stimulating TrkB ubiquitination (Fig. S6). The data presented herein suggest that α-Syn blocks TrkB signaling by diminishing TrkB endocytosis, internalization, and axonal transport. Though α-Syn overexpression in neurons from *SNCA* overexpressing transgenic mice greatly inhibits BDNF/TrkB neurotrophic signaling, the absence of α-Syn in neurons of *SNCA* KO mice has little effects on these events. Here, we observed that α-Syn KO exhibit a slight delay in the kinetics of BDNF-induced phosphorylation of TrkB and its downstream effectors. This delay did not affect the neurotrophic activity of BDNF, as the effects of BDNF treatment were comparable in both wild-type and *SNCA* KO DA neurons in the face of MPP^+-induced neurotoxicity (Fig. 2 *C–F*). These observations indicate that *SNCA* is not required for BDNF/TrkB neurotrophic activities, despite the finding that increased levels of α-Syn can inhibit BDNF neurotrophic signaling. Previous studies indicate that BDNF stimulates the association between endogenous TrkB and Fyn. In neurons derived from Fyn knockout mice, the translocation of TrkB to lipid rafts in response to BDNF is compromised, while inhibiting TrkB translocation to lipid rafts prevents the full activation of TrkB and downstream signals (21). In the current study, we replicated the finding that BDNF treatment translocates TrkB to lipid rafts and extended these findings to show that α-Syn overexpression diminishes TrkB lipid raft residency, whereas α-Syn depletion does not affect TrkB subcellular distribution. Using TrkB stably transfected dopaminergic SH-SY5Y cells, we showed that BDNF-triggered TrkB internalization is abolished by α-Syn overexpression. These findings are consistent with previous reports that BDNF-induced TrkB accumulation at lipid rafts is prevented by blocking the internalization of TrkB (21). Conceivably, BDNF treatment activates Fyn, which subsequently phosphorylates α-Syn on Y125, resulting in a dissociation from TrkB, allowing TrkB lipid raft translocation. α-Syn interacts with complex I of the mitochondrial respiratory chain and interferes with its function, promoting the production of reactive oxygen species (ROS) (25). Accumulating evidence suggests that the toxic interaction between DA, DA metabolites, and α-Syn might promote an oxidative environment within DAergic neurons. Oxidative modification of α-Syn by DA metabolites has been proposed to be responsible for the selective vulnerability to DAergic neurons (26, 27). DA-modified α-Syn tends to form protofibrillar intermediates but not large fibrils (27). Such "oligomeric" α-Syn has been suggested to represent the primary toxic species responsible for DAergic neurotoxicity (28). Consistent with these reports, we found that DA and its metabolite, DOPAL, strongly stimulate α-Syn binding

to TrkB receptors, completely blocking TrkB neurotrophic signaling, a phenomenon which was partially attenuated by BDNF. Accordingly, α-Syn overexpression-induced neuronal cell death was further escalated by DOPAL. Moreover, blocking DA biosynthesis by α-methyl-p-tyrosine (AMPT), which inhibits TH activity, significantly attenuates α-Syn–elicited neuronal cell death (Fig. S3). Elevation of DOPAL levels may contribute to the specific vulnerability of DAaergic neurons to complex I inhibition (29). DOPAL covalently modifies α-Syn and stimulates its aggregation and is neurotoxic in vivo (30). Fitting with in vitro results (Fig. S4), we found that rasagiline strongly dissociates the α-Syn/TrkB complex in the SNpc of mice injected with AAV–α-Syn or MPTP, resulting in a subsequent up-regulation of p-TrkB/p-Akt/p-MAPK signaling. Consequently, rasagiline significantly attenuated α-Syn–induced DAergic neuron death in the SN with the concomitant preservation of DA terminals within the striatum and improvements in motor functions. These findings are consistent with previous reports that rasagiline promotes regeneration of SN dopaminergic neurons in post–MPTP-induced parkinsonism via activation of tyrosine kinase receptor signaling pathway (31, 32).

Here we show that α-Syn binds to, and inhibits, TrkB neurotrophic activities. The current study provides a pathological function for α-Syn in the binding of the TrkB receptor and resultant inhibition of BDNF/TrkB neurotrophic signaling. This interaction is strongly up-regulated by DA's metabolite DOPAL, resulting in increased dopaminergic neuronal death. This discovery provides a model for the underlying molecular etiology of α-Syn–mediated neurotoxicity and DAergic neuronal loss in PD.

Methods

Animals. All mice were obtained from the Jackson Laboratory. Eight- to 12-wk-old C57BL/6 were used as controls. *SNCA* null mice (B6;129 × 1-*Snca*^{tm1Rosl}, stock no. 003692) or human *SNCA* overexpressing mice [B6.Cg-Tg(*SNCA*)OVX37Rwm *Snca*^{tm1Rosl}/J, stock no. 023837] on pure genetic background were backcrossed. Genotyping was performed by PCR using genomic DNA isolated from the tail tip. PCR was performed using mutant primers F (5′TCA GCC ACG ATA AAA CTG AGG3′), R (5′GCC TGA AGA ACG AGA TCA GC3′) and transgene primers F (5′CCT CCT GTT AGC TGG GCT TT3′), R (5′ACC ACT CCC TCC TTG GTT TT3′). Animal care and handling were performed according to the Declaration of Helsinki and Emory Medical School guidelines. Investigators were blinded to the group allocation during the animal experiments. The protocol was reviewed and approved by the Emory Institutional Animal Care and Use Committee.

Human Tissue Samples. Postmortem brain frozen samples of LBD patients ($n = 3$) and paraffin-embedded sections of PD patients ($n = 3$) were provided from the Emory Alzheimer's Disease Research Center. The study was approved by the biospecimen committee at Emory University. LBD and PD cases were clinically diagnosed and neuropathologically confirmed. Informed consent was obtained from all subjects.

Plasmid Clones and Viral Genomes. AAV viral genome contained either the human α-syn or the GFP coding sequence controlled by the hybrid chicken beta-actin/cytomegealovirus promoter. AAV2/5 and LV pseudotyped with VSV-G was produced as described previously (33).

Statistical Analysis. Statistical analysis was performed using either Student's *t* test (two-group comparison) or one-way ANOVA followed by LSD post hoc test (more than two groups), and differences with *P* values less than 0.05 were considered significant.

ACKNOWLEDGMENTS. We thank Alzheimer's Disease Research Center at Emory University for human PD and LBD patients and healthy control samples. This work was supported by M. J. FOX Foundation Grant 11137 and NIH Grant RF1, AG051538 (to K.Y.); National Key Basic Research Program of China Grant 2010CB945202 (to Y.E.S.); and NSFC Grants 81461138037, 31471029, and 31671055 (to J.X.).

1. Polymeropoulos MH, et al. (1997) Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science* 276:2045–2047.
2. Spillantini MG, Crowther RA, Jakes R, Hasegawa M, Goedert M (1998) Alpha-synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with Lewy bodies. *Proc Natl Acad Sci USA* 95:6469–6473.
3. Goedert M (2001) Alpha-synuclein and neurodegenerative diseases. *Nat Rev Neurosci* 2:492–501.
4. Lotharius J, Brundin P (2002) Pathogenesis of Parkinson's disease: Dopamine, vesicles and alpha-synuclein. *Nat Rev Neurosci* 3:932–942.
5. Krüger R, et al. (1998) Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. *Nat Genet* 18:106–108.
6. Zarranz JJ, et al. (2004) The new mutation, E46K, of alpha-synuclein causes Parkinson and Lewy body dementia. *Ann Neurol* 55:164–173.
7. Simón-Sánchez J, et al. (2009) Genome-wide association study reveals genetic risk underlying Parkinson's disease. *Nat Genet* 41:1308–1312.
8. Yasuda T, Nakata Y, Mochizuki H (2013) α-Synuclein and neuronal cell death. *Mol Neurobiol* 47:466–483.
9. Huang EJ, Reichardt LF (2003) Trk receptors: Roles in neuronal signal transduction. *Annu Rev Biochem* 72:609–642.
10. Seroogy KB, et al. (1994) Dopaminergic neurons in rat ventral midbrain express brain-derived neurotrophic factor and neurotrophin-3 mRNAs. *J Comp Neurol* 342:321–334.
11. Hagg T (1998) Neurotrophins prevent death and differentially affect tyrosine hydroxylase of adult rat nigrostriatal neurons in vivo. *Exp Neurol* 149:183–192.
12. Parain K, et al. (1999) Reduced expression of brain-derived neurotrophic factor protein in Parkinson's disease substantia nigra. *Neuroreport* 10:557–561.
13. Mogi M, et al. (1999) Brain-derived growth factor and nerve growth factor concentrations are decreased in the substantia nigra in Parkinson's disease. *Neurosci Lett* 270:45–48.
14. Howells DW, et al. (2000) Reduced BDNF mRNA expression in the Parkinson's disease substantia nigra. *Exp Neurol* 166:127–135.
15. Molina-Holgado F, Doherty P, Williams G (2008) Tandem repeat peptide strategy for the design of neurotrophic factor mimetics. *CNS Neurol Disord Drug Targets* 7: 110–119.
16. Altar CA, et al. (1992) Brain-derived neurotrophic factor augments rotational behavior and nigrostriatal dopamine turnover in vivo. *Proc Natl Acad Sci USA* 89: 11347–11351.
17. Klein RL, Lewis MH, Muzyczka N, Meyer EM (1999) Prevention of 6-hydroxydopamine-induced rotational behavior by BDNF somatic gene transfer. *Brain Res* 847:314–320.
18. Kohno R, et al. (2004) BDNF is induced by wild-type alpha-synuclein but not by the two mutants, A30P or A53T, in glioma cell line. *Biochem Biophys Res Commun* 318: 113–118.
19. von Bohlen und Halbach O, Minichiello L, Unsicker K (2005) Haploinsufficiency for trkB and trkC receptors induces cell loss and accumulation of alpha-synuclein in the substantia nigra. *FASEB J* 19:1740–1742.
20. Yuan Y, et al. (2010) Overexpression of alpha-synuclein down-regulates BDNF expression. *Cell Mol Neurobiol* 30:939–946.
21. Pereira DB, Chao MV (2007) The tyrosine kinase Fyn determines the localization of TrkB receptors in lipid rafts. *J Neurosci* 27:4859–4869.
22. Grimes ML, et al. (1996) Endocytosis of activated TrkA: Evidence that nerve growth factor induces formation of signaling endosomes. *J Neurosci* 16:7950–7964.
23. Beattie EC, Howe CL, Wilde A, Brodsky FM, Mobley WC (2000) NGF signals through TrkA to increase clathrin at the plasma membrane and enhance clathrin-mediated membrane trafficking. *J Neurosci* 20:7325–7333.
24. Volpicelli-Daley LA, et al. (2014) Formation of α-synuclein Lewy neurite-like aggregates in axons impedes the transport of distinct endosomes. *Mol Biol Cell* 25: 4010–4023.
25. Devi L, Raghavendran V, Prabhu BM, Avadhani NG, Anandatheerthavarada HK (2008) Mitochondrial import and accumulation of alpha-synuclein impair complex I in human dopaminergic neuronal cultures and Parkinson disease brain. *J Biol Chem* 283: 9089–9100.
26. Xu J, et al. (2002) Dopamine-dependent neurotoxicity of alpha-synuclein: A mechanism for selective neurodegeneration in Parkinson disease. *Nat Med* 8:600–606.
27. Conway KA, Rochet JC, Bieganski RM, Lansbury PT, Jr (2001) Kinetic stabilization of the alpha-synuclein protofibril by a dopamine-alpha-synuclein adduct. *Science* 294: 1346–1349.
28. Winner B, et al. (2011) In vivo demonstration that alpha-synuclein oligomers are toxic. *Proc Natl Acad Sci USA* 108:4194–4199.
29. Lamensdorf I, et al. (2000) 3,4-Dihydroxyphenylacetaldehyde potentiates the toxic effects of metabolic stress in PC12 cells. *Brain Res* 868:191–201.
30. Burke WJ, et al. (2008) Aggregation of alpha-synuclein by DOPAL, the monoamine oxidase metabolite of dopamine. *Acta Neuropathol* 115:193–203.
31. Mandel SA, Sagi Y, Amit T (2007) Rasagiline promotes regeneration of substantia nigra dopaminergic neurons in post-MPTP-induced parkinsonism via activation of tyrosine kinase receptor signaling pathway. *Neurochem Res* 32:1694–1699.
32. Weinreb O, Amit T, Bar-Am O, Youdim MB (2007) Induction of neurotrophic factors GDNF and BDNF associated with the mechanism of neurorescue action of rasagiline and ladostigil: New insights and implications for therapy. *Ann N Y Acad Sci* 1122: 155–168.
33. Benskey MJ, Sandoval IM, Manfredsson FP (2016) Continuous collection of adeno-associated virus from producer cell medium significantly increases total viral yield. *Hum Gene Ther Methods* 27:32–45.