RESEARCH ARTICLE

Morpho-Functional Changes of Nigral Dopamine Neurons in an α-Synuclein Model of Parkinson's Disease

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ABSTRACT: Background: The accumulation of α-synuclein (α-syn) fibrils in intraneuronal inclusions called Lewy bodies and Lewy neurites is a pathological signature of Parkinson's disease (PD). Although several aspects linked to α-syn–dependent pathology (concerning its spreading, aggregation, and activation of inflammatory and neurodegenerative processes) have been under intense investigation, less attention has been devoted to the real impact of α-syn overexpression on structural and functional properties of substantia nigra pars compacta (SNpc) dopamine (DA) neurons, particularly at tardive stages of α-syn buildup, despite this has obvious relevance to comprehending mechanisms beyond PD progression.

Objectives: We aimed to determine the consequences of a prolonged α-syn overexpression on somatodendritic morphology and functions of SNpc DA neurons.

Methods: We performed immunohistochemistry, stereological DA cell counts, analyses of dendritic arborization, ex vivo patch-clamp recordings, and in vivo DA microdialysis measurements in a 12- to 13-month-old transgenic rat model overexpressing the full-length human α-syn (*Snca*$^{+/+}$) and age-matched wild-type rats.

Results: Aged *Snca*$^{+/+}$ rats have mild loss of SNpc DA neurons and decreased basal DA levels in the SN. Residual nigral DA neurons display smaller soma and compromised dendritic arborization and, in parallel, increased firing activity, switch in firing mode, and hyperexcitability associated with hypofunction of fast activating/inactivating voltage-gated K^+ channels and Ca^{2+}- and voltage-activated large conductance K^+ channels. These intrinsic currents underlie the repolarization/afterhyperpolarization phase of action potentials, thus affecting neuronal excitability.

Conclusions: Besides clarifying α-syn–induced pathological landmarks, such evidence reveals compensatory functional mechanisms that nigral DA neurons could adopt during PD progression to counteract neurodegeneration. © 2022 The Authors. *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society.

Key Words: α-synuclein; substantia nigra; dopamine; firing activity; dendritic arborization; Parkinson's disease

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The presence of α-synuclein (α-syn) aggregates known as Lewy bodies and Lewy neurites is a pathological hallmark of Parkinson's disease (PD).[1,2] Increasing evidence suggests that abnormal α-syn proteostasis contributes to PD pathogenesis and progression.[3-5] α-Syn mutations are determinants of familial PD,[6] and the overexpression of nonmutated α-syn is linked to PD.[7,8] Moreover, PD-related pathological features have been reported in several animal models obtained by expression or overexpression of either mutated or nonmutated forms of α-syn or following intrastriatal injection of α-syn preformed fibrils.[9-16] Although the diffusion of α-syn aggregates outside the nigrostriatal dopaminergic circuit appears mainly involved in nonmotor symptoms of PD (ie, olfactory, autonomic and sleep dysfunctions, anxiety, fear),[17,18] α-syn–related pathological changes in substantia nigra pars compacta (SNpc) dopamine (DA) neurons are supposed to be primarily liable for PD motor dysfunctions. Despite intense investigation that clarified some aspects linked to α-syn–dependent pathology, namely, those concerning fibril aggregation, spreading, and potential contribution to inflammatory and neurodegenerative processes,[3] the precise mechanisms by which abnormal α-syn lead to PD pathology are largely undefined, and the understanding of the impact of α-syn overload on the functional properties of SNpc DA neurons is still initial.[19] Some studies have reported functional changes in nigral DA neurons during acute α-syn exposure or early stages of α-syn fibril accumulation.[15,19-24] The emerging picture supports that α-syn accumulation perturbs SNpc DA neurons' activity in a bidirectional and time-dependent manner.[19] Beyond this evidence focused on the early α-syn–induced effect, adaptations occurring in nigral DA neurons at late time-points of α-syn accumulation remain less described. Thus, we aimed to extend the understanding of α-syn–dependent pathology to a more advanced stage to unravel the functional adjustments that nigral DA neurons adopt to cope with the α-syn overload. To this aim, we used a transgenic PD rat model overexpressing the full-length human α-syn (*Snca*$^{+/+}$ rats) and leading to the expression of multiple isoforms of the human *Snca*[25] in rat brains expressing the endogenous *Snca* gene.[14] In this model, we investigated the effects of α-syn accumulation on somatodendritic morphology and functional properties of SNpc DA neurons of 12- to 13-month-old rats. Our results might be instructive in unveiling the cellular changes underlying PD pathology and might open to novel therapeutic targets to treat PD symptoms and/or progression.

Methods

Detailed methodological information is provided in the Supplementary Methods S1.

Results

α-Syn Overexpression Induces Somatodendritic Alterations in SNpc DA Neurons

To evaluate the histopathological effects caused by a prolonged α-syn overload on nigral DA neurons, we first estimated SNpc DA neuron loss by stereological cell counts of tyrosine hydroxylase (TH)-expressing neurons in 12- to 13-month-old *Snca*$^{+/+}$ and wild-type (WT) rats. We found a reduced number of TH$^+$ neurons, but not of TH$^-$ cells, in the SNpc of *Snca*$^{+/+}$ in comparison with WT rats (Fig. 1A) along with equal amounts of TH$^+$ neurons in the ventral tegmental area (VTA) (Fig. 1B), the adjacent DA nucleus that remains spared during the initial stages of PD progression. This supports a preferential vulnerability of nigral DA neurons under prolonged α-syn overexpression, in line with recent evidence in the same-aged rat model.[26] We next investigated the somatic morphology of SNpc TH$^+$ neurons, unveiling alterations in soma size (namely, decreased perimeter and area) in *Snca*$^{+/+}$ rats compared with WT rats (Fig. 1C). Then we examined TH$^+$ dendrites of SNpc DA neurons extending ventrally to the substantia nigra pars reticulata (SNpr), forming extensive dendritic arborizations. Aged *Snca*$^{+/+}$ rats displayed a substantial reduction in the TH optical density within the SNpr (Fig. 1D), thus proving that α-syn accumulation impairs DA neurons' dendritic projections to the SNpr. To better analyze the integrity of dendritic arborization, we dialyzed single SNpc DA neurons with the neurotracer biocytin by using the patch-clamp technique on midbrain slices[27] from *Snca*$^{+/+}$ and WT rats. Dendritic arbor of SNpc DA neurons loaded with biocytin (5 mM in the intracellular solution) was visualized by confocal microscopy and, after three-dimensional (3D) reconstruction, was analyzed with 3D Sholl and skeleton analyses. We found that SNpc DA neurons from aged *Snca*$^{+/+}$ rats exhibited compromised dendritic branching, displaying less dendritic intersections to increased radial distance from the soma (Fig. 2A,B), reduced cumulative dendritic length (Fig. 2C), and fewer dendritic endpoints for each cell (Fig. 2C). Notably, nigral DA neurons at an earlier time-point of α-syn overexpression (as in 5-month-old *Snca*$^{+/+}$ rats) had preserved dendritic arborization (Fig. S1), thus indicating that α-syn burden produces progressive impairment of the dendritic arbor of nigral DA neurons.

SNpc DA neurons release DA either at the somatodendritic level or at terminal projections in the dorsal striatum.[28,29] Although previous evidence documented α-syn–induced alterations in striatal DA release, the impact of α-syn overexpression on the somatodendritic DA release within the substantia nigra (SN) has not been explored yet. Thus, we directly measured extracellular DA basal levels in SN via

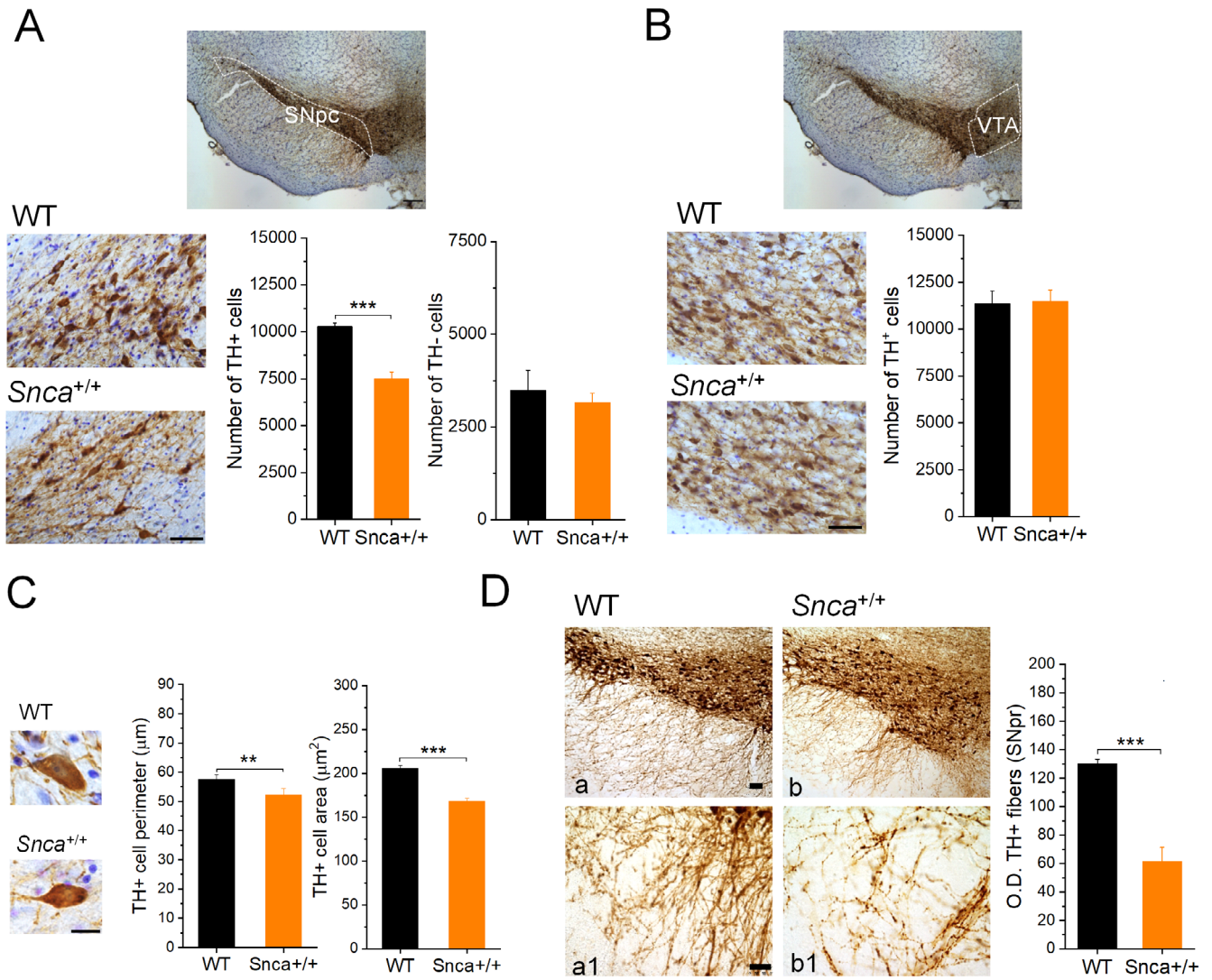

FIG. 1. α-Synuclein overexpression causes somatodendritic morphological alterations in substantia nigra pars compacta (SNpc) dopamine (DA) neurons. (A) Coronal midbrain slice showing the SNpc area analyzed in the stereological cell counts (scale bar: 200 µm) (top), SNpc microphotographs obtained from 12- to 13-month-old wild-type (WT) and *Snca*$^{+/+}$ rats (scale bar: 20 µm) (left), and bar plot of stereological quantification of tyrosine hydroxylase (TH)$^{+}$ or TH^{-} neurons in SNpc showing a reduced number of TH^{+} neurons in *Snca*$^{+/+}$ rats compared with WT rats ($n = 4$ rats/group; SNpc TH^{+} neurons: t test, $t = -7.149$; $P = 3.77 \times 10^{-4}$) (right). (B) Coronal midbrain slice showing the ventral tegmental area (VTA) zone analyzed in the stereological cell counts (scale bar: 200 µm) (top), microphotographs of VTA from 12- to 13-month-old WT and *Snca*$^{+/+}$ rats (scale bar: 20 µm) (left), and plot of VTA TH^{+} neurons ($n = 4$ rats/group; $P > 0.05$) (right). (C) High magnification images of SNpc TH^{+} DA neurons from 12- to 13-month-old WT and *Snca*$^{+/+}$ rats showing reduced soma size of *Snca*$^{+/+}$ DA neurons (scale bar: 10 µm) (left) and bar plots indicating mean values of TH^{+} cell soma perimeter (µm; t test, $t = 4.376$; $P = 0.004$) and soma area (µm^2; t test, $t = 8.450$; $P = 1.4 \times 10^{-4}$) measured from 50 neurons/group ($n = 4$ rats/group) (right). (D) Representative images of midbrain slices containing SNpc and substantia nigra pars reticulata (SNpr) obtained from 12- to 13-month-old WT and *Snca*$^{+/+}$ rats (a, b) and respective high magnification of SNr fields (a1, b1) showing the decreased density of TH^{+} dendrites spreading from SNpc to SNpr in *Snca*$^{+/+}$ compared with WT rats (left) (scale bar: 100 µm [a, b] and 10 µm [a1, b1]) and graph of mean optical density (O.D.) of TH staining of dendrites within SNpr measured in *Snca*$^{+/+}$ and WT midbrain slices ($n = 6$ rats/group; t test, $t = 9.563$; $P = 1.526 \times 10^{-11}$) (right). (A-D) *$P < 0.01$; ** $P < 0.001$.

microdialysis experiments in freely moving rats. We found that aged *Snca*$^{+/+}$ rats had reduced basal DA levels in SN compared with WT rats (Fig. 2D).

α-Syn Overexpression Perturbs Firing Activity of SNpc DA Neurons

To explore the effects of prolonged α-syn overload on the functional properties of SNpc DAergic neurons, we performed electrophysiological recordings in acute midbrain slices from 12- to 13-month-old *Snca*$^{+/+}$ and WT rats, examining DA neurons' spontaneous firing activity in the patch-clamp cell-attached configuration. In aged WT rats, in addition to spontaneously active DA neurons (firing as a regular pacemaker or bursting mode),[30] we found a population of silent DA neurons. Precisely, the majority of WT DA neurons was silent (56.41%, 22/39 cells), one-third was regular spiking (33.33%, 13/39 cells), and a minor population fired in

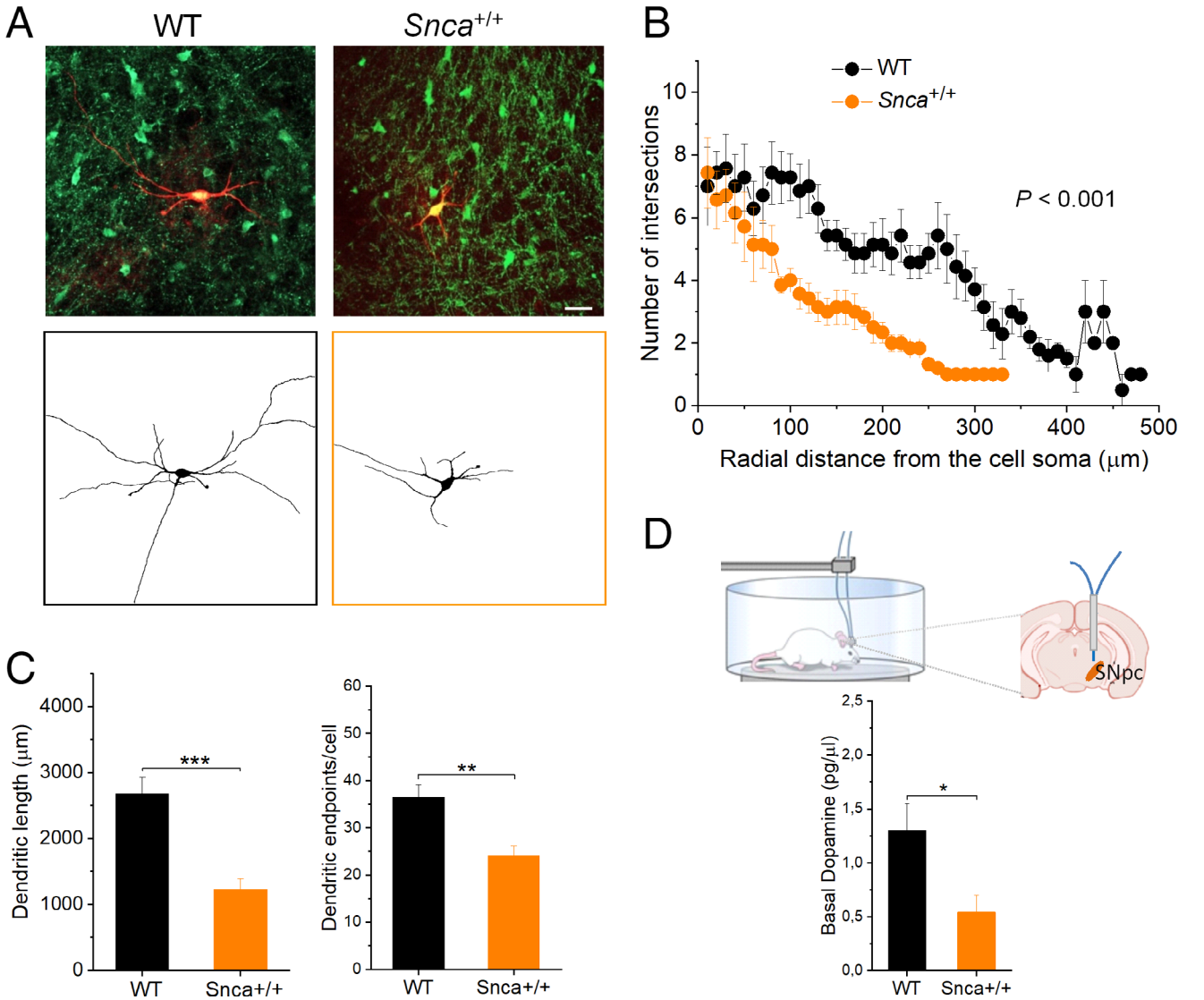

FIG. 2. α-Synuclein overexpression damages dendritic arborization of nigral dopamine (DA) neurons and reduces basal DA levels in the substantia nigra. (A) Confocal images of substantia nigra pars compacta (SNpc) slices from 12- to 13-month-old wild-type (WT) and *Snca*$^{+/+}$ rats stained with tyrosine hydroxylase (TH) (green) showing single biocytin-filled nigral DA neurons surrounded by numerous TH^{+} neurons (scale bar: 200 μm) (top) and three-dimensional morphological reconstruction of soma and proximal dendrites of the biocytin-filled DA neurons (bottom). (B) Plot of the mean number of intersections along the radial distance from the neuronal soma indicating reduced dendritic branching of SNpc DA neurons from 12- to 13-month-old *Snca*$^{+/+}$ rats compared with WT (WT: $n = 7$ neurons/3 rats, and *Snca*$^{+/+}$ $n = 7$ neurons/3 rats; one-way analysis of variance repeated-measures followed by Bonferroni's test, ***$P < 0.001$). (C) Bar plot indicating that mean dendritic length (μm) is reduced in SNpc DA neurons from *Snca*$^{+/+}$ compared with WT rats ($n = 7$ neurons/3 rats for each group; t test, $t = 4.957$; $P = 3.324 \times 10^{-4}$) (left) and graph of the mean number of dendritic endpoints/cells showing minor dendritic branches in nigral DA neurons from *Snca*$^{+/+}$ rats with respect to WT rats ($n = 7$ neurons/3 rats for each group; t test, $t = 3.742$; $P = 0.0028$) (right). **$P < 0.01$; ***$P < 0.001$. (D) Scheme depicting in vivo microdialysis experiments in freely moving rats and a coronal slice indicating probe placement in the SNpc (top) and plot showing the reduction of basal DA concentration (pg/μl) in the SNpc of 12- to 13-month-old *Snca*$^{+/+}$ rats ($n = 6$) compared with age-matched WT rats ($n = 5$) (t test, $t = -2.703$; $P = 0.0242$) (bottom). *$P < 0.05$.

bursting mode (10.25%, 4/39 cells) (Fig. 3A). Interestingly, α-syn overexpression subverted the intrinsic firing properties of DA neurons. Indeed, in aged *Snca*$^{+/+}$ rats, the preponderance of DA neurons was spontaneously active, with an increased occurrence of either regular spiking (63.63%, 21/33 cells) or bursting neurons (24.24%, 8/33 cells) associated with a significant reduction of silent cells (12.12%, 4/33 cells) (Fig. 3A). These data indicate a clear-cut shift toward an increase in spontaneous firing and hyperactivity of SNpc DA neurons in aged *Snca*$^{+/+}$ rats. Furthermore, in whole-cell mode, SNpc DA neurons from *Snca*$^{+/+}$ rats displayed more action potentials evoked by current injections than those from WT rats (Fig. 3B). α-Syn overexpression also altered the passive properties of SNpc DA neurons, causing an increase in the membrane input

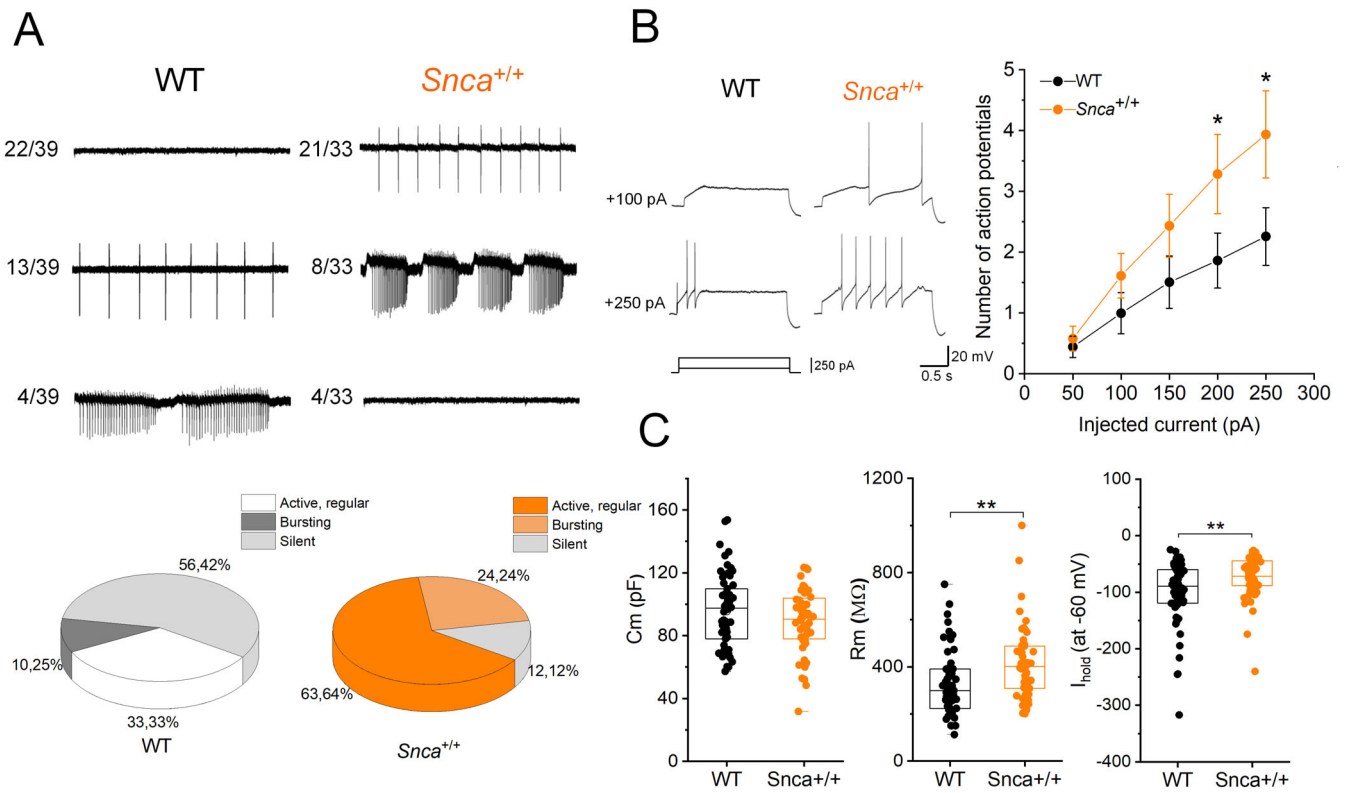

FIG. 3. α-Synuclein overexpression perturbs firing activity of substantia nigra pars compacta (SNpc) dopamine (DA) neurons. (A) Example traces of spontaneous firing activity in cell-attached recordings of SNpc DA neurons from 12- to 13-month-old wild-type (WT) and *Snca*$^{+/+}$ rats (top). Incidence of silent, firing-pacemaker, and firing-bursting neurons is reported aside from each example trace as the number of cells showing the indicated firing mode/total number of recorded neurons. Pie diagrams show the percentage of SNpc DA neurons in the different firing modes (bottom). (B) Representative traces of action potentials (APs) evoked by current injections (+50/+250 pA, holding voltage (V_H) = −60 mV, 2 seconds) in the SNpc DA neurons of WT and *Snca*$^{+/+}$ rats (scale bar: 20 mV/0.5 seconds) (left) and plot of mean APs evoked at each current step (WT n_{cells} = 42/6 rats, *Snca*$^{+/+}$ n_{cells} = 41/6 rats; two-way repeated measures (RM) analysis of variance (ANOVA) followed by Tukey's test, stimulus intensity × genotype interaction, $F = 4.278$ and $P = 0.028$; $t = 4.143$ and $P = 0.003$ at 200 pA; and $t = 4.869$ and $P = 7.339 \times 10^{-4}$ at 250 pA) (right). (C) Box plots showing passive properties (membrane capacitance (C_m), membrane input resistance (R_m), and holding current (I_{hold}) to −60 mV) of *Snca*$^{+/+}$ and WT SNpc DA neurons (WT n_{cells} = 57/6 rats and *Snca*$^{+/+}$ n_{cells} = 52/6 rats, C_m: *t* test, $t = 1.692$, $P = 0.093$; R_m: Mann–Whitney test, $U = 927$, $P = 0.0017$; I_{hold} to −60 mV: Mann–Whitney test, $U = 1917$, $P = 0.0079$).

resistance (R_m) and a reduction of the current to maintain holding voltage (V_H) at −60 mV (Fig. 3C).

α-Syn Overexpression Affects Intrinsic Conductance of SNpc DA Neurons

To identify the functional mechanisms by which α-syn overload affects spontaneous firing and excitability of SNpc DA neurons, we analyzed various intrinsic currents known to regulate their pacemaker activity and firing rate fidelity.[31] First, we examined the hyperpolarization-activated current (I_h) mediated by hyperpolarization-activated cyclic nucleotide-gated (HCN) channels,[32,33] but we did not find differences in I_h peak amplitude (Fig. 4A) or I_h tail conductance (Fig. S2) between *Snca*$^{+/+}$ and WT SNpc DA neurons. We next analyzed depolarization-gated K^+ channel-mediated currents (I_{Kv}), revealing that the peak current (fast activating/inactivating A-type K^+ current, I_A[31,34,35]) was reduced in SNpc DA neurons of *Snca*$^{+/+}$ rats compared with WT rats while the steady-state current was unaffected (Fig. 4B). This suggests that prolonged α-syn overexpression selectively impairs A-type K^+ channels, in line with previous evidence from nigral DA neurons of mutated A53T-*Snca* overexpressing mice.[21]

Then, we evaluated the afterhyperpolarization-associated current (I_{AHP}), which underlies the afterhyperpolarization phase of the action potential,[31,36-38] and found that nigral DA neurons of aged *Snca*$^{+/+}$ rats have reduced I_{AHP} compared with those of WT rats, with changes in I_{AHP} peak (and area) (Fig. 4C,D) overt in different intracellular Ca^{2+} buffering conditions, namely, in the presence of 0.75 mM (Fig. 4C) or 0.1 mM ethylene glycol tetraacetic acid (EGTA) (Fig. 4D). Different types of Ca^{2+}-activated K^+ channels mediate I_{AHP}, that is, small conductance K^+ (SK) channels, which are purely Ca^{2+} activated, and big conductance K^+ (BK) channels, whose activation is either voltage or Ca^{2+} dependent.[31] To determine the channel subtype(s) underlying the I_{AHP} reduction caused by prolonged α-syn overexpression, we measured I_{AHP} (in 0.1 mM EGTA) before and during

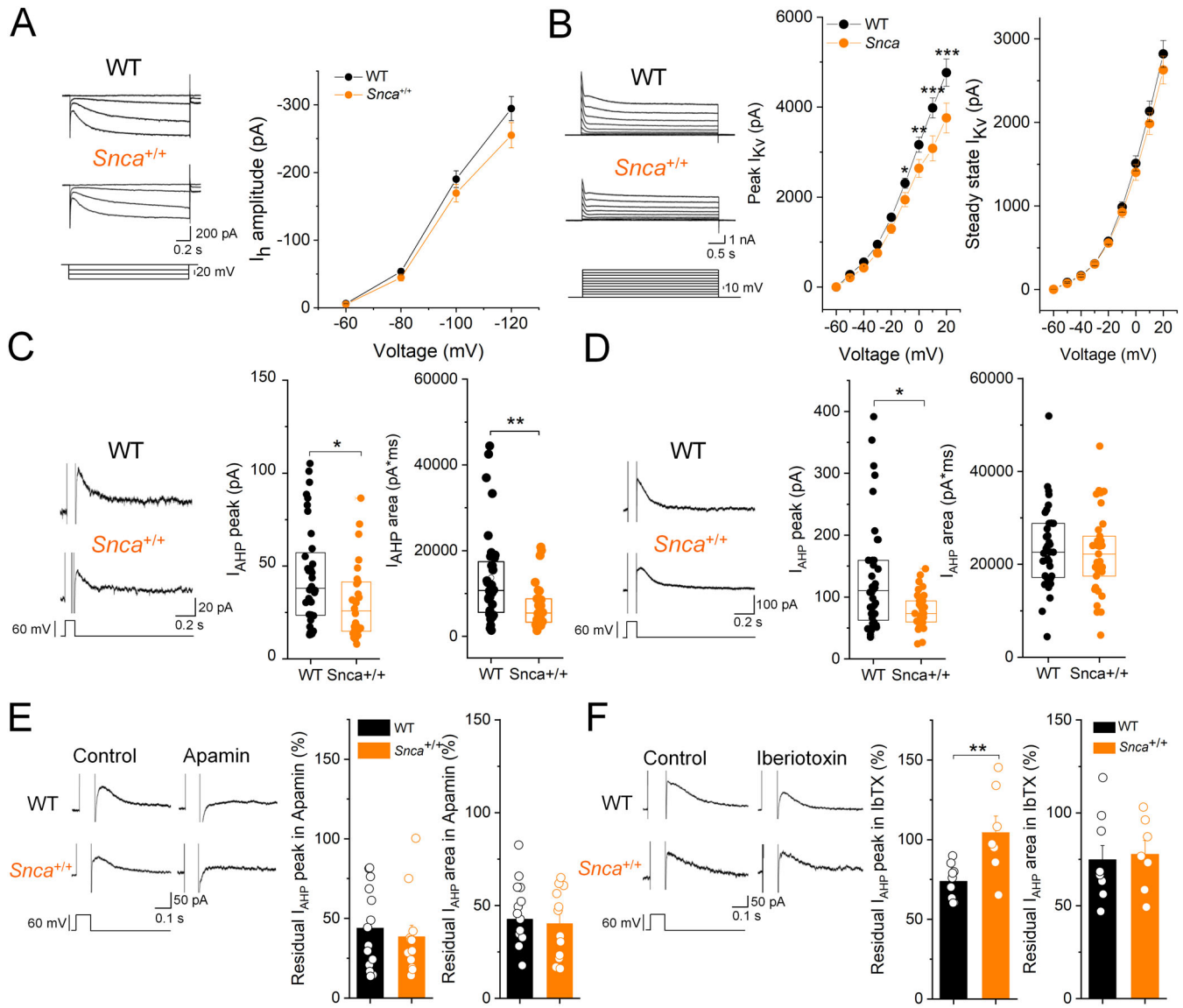

FIG. 4. Prolonged α-synuclein (α-syn) overexpression causes hypofunction of voltage-gated K^+ channels and big conductance K^+ (BK) channels in nigral dopamine (DA) neurons. (A) Example traces of hyperpolarization-activated current (I_h) elicited by voltage steps (−60 to −120 mV, holding voltage (V_H) = −60 mV) in wild-type (WT) and *Snca*$^{+/+}$ substantia nigra pars compacta (SNpc) DA neurons (scale bar: 200 pA/0.2 seconds) (left) and graph of I_h amplitudes at different voltage steps (WT n_{cells} = 57/6 rats; *Snca*$^{+/+}$ n_{cells} = 45/6 rats; two-way repeated measures (RM) analysis of variance (ANOVA), $P > 0.05$) (right). (B) Example traces of voltage-gated K^+ channels-mediated currents (I_{Kv}) elicited by depolarizing steps (−60 mV/+20 mV, V_H = −60 mV) in SNpc DA neurons (left) and plots of peak and steady-state I_{Kv} showing that *Snca*$^{+/+}$ DA neurons have reduced peak I_{Kv} compared with WT (WT n_{cells} = 15/3 rats; *Snca*$^{+/+}$ n_{cells} = 17/3 rats; two-way RM ANOVA followed by Tukey's test; peak I_{Kv}: voltage × genotype interaction: $F = 3.037$ and $P = 0.005$; $t = 3.563$ and $P = 0.013$ at −10 mV; $t = 4.694$ and $P = 0.001$ at 0 mV; $t = 5.573$ and $P = 1.860 \times 10{-}4$ at +10 mV; $t = 6.219$ and $P = 3.71 \times 10{-}5$ at +20 mV) (right). $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. (C) Example traces of afterhyperpolarization-associated currents (I_{AHP}) elicited in SNpc DA neurons by a 100-millisecond voltage step to 0 mV (V_H = −60 mV) in 0.75 mM ethylene glycol tetraacetic acid (EGTA) (scale bar: 20 pA/0.2 seconds) (left) and box plots show a reduction of I_{AHP} peak and area in *Snca*$^{+/+}$ DA neurons compared with WT (WT n_{cells} = 36/6 rats and *Snca*$^{+/+}$ n_{cells} = 26/6 rats; I_{AHP} peak: Mann–Whitney test, $U = 313$, $P = 0.026$; I_{AHP} area: Mann–Whitney test; $U = 671$, $P = 0.003$) (right). $^*P < 0.05$; $^{**}P < 0.01$. (D) Example I_{AHP} traces in WT and *Snca*$^{+/+}$ SNpc DA neurons in 0.1 mM EGTA (scale bar: 100 pA/0.2 seconds) (left) and box plots show a reduction of I_{AHP} peak in *Snca*$^{+/+}$ DA neurons compared with WT (WT n_{cells} = 40/7 rats and *Snca*$^{+/+}$ n_{cells} = 33/7 rats; I_{AHP} peak: Mann–Whitney test, $U = 878$, $P = 0.015$; I_{AHP} area: Mann–Whitney test; $U = 700$, $P = 0.43$) (right). $^*P < 0.05$. (E) Effect of the small conductance K^+ (SK) blocker apamin on I_{AHP} recorded in *Snca*$^{+/+}$ and WT nigral DA neurons (in 0.1 mM EGTA). Example I_{AHP} traces before and during apamin (100 nM) (left) and plots of the residual I_{AHP} peak and area in apamin (as percentage of control I_{AHP}) demonstrating that SK channels similarly contribute to I_{AHP} in *Snca*$^{+/+}$ and WT DA neurons (WT n_{cells} = 14/3 rats and *Snca*$^{+/+}$ n_{cells} = 12/3 rats, I_{AHP} peak: Mann-Whithey test, $U = 93$, $P = 0.667$; I_{AHP} area, Mann-Whitney test, $U = 93$, $P = 0.668$) (scale bar: 50 pA/0.1 seconds) (right). (F) Effect of the BK blocker iberiotoxin (IbTX) on I_{AHP} recorded in *Snca*$^{+/+}$ and WT DA neurons (in 0.1 mM EGTA). Example I_{AHP} traces before and during IbTX (100 nM) (left) and plots of the residual I_{AHP} peak and area in IbTX (as percentage of control I_{AHP}) (right). Of note, the IbTX-induced I_{AHP} reduction is occluded in DA neurons from *Snca*$^{+/+}$ rats, indicating that BK channel hypofunction occurs during prolonged α-syn overexpression (WT n_{cells} = 9/3 rats and *Snca*$^{+/+}$ n_{cells} = 7/3 rats, I_{AHP} peak: *t* test, $t = -3.055$, $P = 0.008$; I_{AHP} area: *t* test, $t = 0.275$, $P = 0.787$). Scale bar: 50 pA/0.1 seconds. $^{**}P < 0.01$.

the application of the selective SK blocker apamin (100 nM) or the BK blocker iberiotoxin (IbTX) (100 nM). Although apamin similarly reduced I_{AHP} in nigral DA neurons from aged *Snca*$^{+/+}$ and WT rats, indicating equal SK channel contribution to total I_{AHP} in the two genotypes (Fig. 4E), the IbTX-sensitive I_{AHP} component in nigral DA neurons was significantly different between the *Snca*$^{+/+}$ and WT rats. IbTX-induced I_{AHP} reduction was occluded in *Snca*$^{+/+}$ rats (Fig. 4F), suggesting that prolonged α-syn overexpression selectively affects BK channel function, causing I_{AHP} reduction in nigral DA neurons.

In conclusion, prolonged α-syn burden impairs different K^+ channel subtypes (BK and A-type K_v) that shape repolarization/afterhyperpolarization phases of action potentials, thus controlling spontaneous firing activity and excitability of nigral DA neurons.[31,35,38-40]

Discussion

In this article, we described morphological and functional alterations occurring in the somatodendritic compartment of SNpc DA neurons in aged rats overexpressing human α-syn. We show that during emergent α-syn–dependent neurodegeneration (with overt signs of damaged dendritic arborization, neuronal soma shrinkage, cell loss, and reduced nigral DA levels), the surviving DA neurons adapt by increasing firing activity, with a switch in the firing mode and hyperexcitability possibly reliant on a hypofunction of voltage-gated K^+ channels and Ca^{2+}-activated and voltage-activated BK channels.

Somatodendritic Pathology and Cell Loss

Substantial evidence from patients and animal models supports that α-syn proteostasis has a central role in PD pathogenesis. It is recognized that α-syn mutations are determinants of familial PD[6] and that overexpression of nonmutated α-syn is linked to PD.[7,8] Moreover, proteinaceous deposits of misfolded and aggregated forms of α-syn (Lewy bodies and Lewy dendrites) are found in the ventral midbrain and other affected brain areas in patients with PD.[1,2,41] Accordingly, PD-related pathological features have been demonstrated in several animal models by expression or overexpression of either mutated or nonmutated forms of α-syn and by local intrastriatal injection of α-syn preformed fibrils.[9-16] However, despite intense investigation, the comprehension of precise mechanisms by which abnormal α-syn levels lead to PD is still incomplete.

In the α-syn overexpressing rat model we used in the present study (*Snca*$^{+/+}$ rats), nigral synucleinopathy has already been demonstrated.[14,23,26] It has been reported that *Snca*$^{+/+}$ rats display (1) age-dependent accumulation of oligomeric and truncated α-syn in various brain areas, including the prefrontal cortex, hippocampus, striatum, and midbrain[14,26]; (2) proteinase K-resistant α-synuclein immunoreactive granules in the perinuclear region of SNpc neurons[14]; and (3) intense labeling of serine129-phosphorylated α-syn (pser129 α-syn) in intracellular aggregates in the SNpc DA neurons at 12 months of age.[26] Thus, *Snca*$^{+/+}$ rats represent a validated PD model to study the early and late consequences of α-syn burden on nigral DA neurons. Finally, although the *Snca*$^{+/+}$ rats present all regulatory elements of the human gene in the rat genome and the model could allow the investigation of SNCA gene expression in vivo, more work is still needed to validate the precise regulatory mechanisms of the human gene that apply in the rat genome.

SNpc DA neurons have extensive dendritic arborizations, with approximately 245,000 estimated release sites for each cell.[42-44] This dendritic complexity, demanding a massive energetic depletion, is a significant vulnerability factor for DA cells.[43,45,46] Evidence in PD animal models and postmortem PD human tissues proves that SNpc DA neurons have decreased dendritic arborization and reduced soma size before neuronal death.[43,45-47] Accordingly, in 12- to 13-month-old *Snca*$^{+/+}$ rats, we show an overt impairment of dendritic arborization and soma shrinkage associated with an incipient neuronal loss (approximately 25% reduction of nigral TH^+ neurons). Noteworthy, in line with the absence of other signs of degeneration,[23] the dendritic arbor of DA cells is preserved at an earlier time-point (5-month-old *Snca*$^{+/+}$ rats). Interestingly, a recent study in neuronal cultures of midbrain DA neurons also demonstrated that virally induced α-syn–overexpressing neurons exhibit a lower degree of dendritic arborization compared with naive DA neurons.[48] Altogether, α-syn–dependent somatodendritic alterations observed in aged *Snca*$^{+/+}$ nigral DA neurons model the pathological changes of the DAergic system occurring in humans during PD progression[43,45-47] and provide additional insights into the mechanisms by which α-syn overload drives SNpc DA neurons' pathology.

Reduced DA Level in SN

In parallel with the described morphological alterations, α-syn overexpression also causes functional alterations of nigral DA neurons. Using in vivo microdialysis experiments in freely moving rats, we demonstrated that α-syn overexpression correlates with a reduction of basal DA levels within the SN. Such a decrease in the somatodendritic DA release may be the consequence of the lower dendritic arborization of SNpc DA neurons of aged *Snca*$^{+/+}$ rats, resulting in fewer active sites of DA release on the dendritic tree. This process may also

summate to additional mechanisms by which α-syn can affect DA neurotransmission,[49] including α-syn–induced inhibition of TH expression and activity (affecting DA synthesis)[50,51] and α-syn–dependent regulation of dopamine transporter expression at the plasma membrane (affecting DA reuptake).[52-54]

Functional Alterations in SNpc DA Neurons

As the real impact of prolonged α-syn accumulation on the functional properties of SNpc DA neurons is not yet entirely known, in this study, beyond the morphological alterations, we aimed at defining the functional changes occurring in nigral DA neurons during persistent α-syn overload.

The spontaneous firing activity of SNpc DA neurons is tightly controlled by several intrinsic and extrinsic factors necessary for generating and homeostatically tuning their activity patterns to physiological demands.[31] Pathological disruption of such fine control, affecting DA levels in the projection areas, can unhinge DA-dependent functions and cause the motor and nonmotor symptoms of PD. In this perspective, clarifying the functional consequences of progressive α-syn accumulation on SNpc DA firing activity is essential to fully comprehending cellular processes governing PD development and progression. Here, we show that prolonged α-syn burden, as it occurs in 12- to 13-month-old *Snca*$^{+/+}$ rats, perturbs spontaneous firing patterns of SNpc DA neurons. Although nigral DA neurons from aged WT rats were predominantly silent (and this, in our opinion, could be reliant on aging-associated processes that could decrease ion channel conductances associated with firing),[55,56] SNpc DA neurons in aged *Snca*$^{+/+}$ rats displayed a shift toward increased activity and bursting mode and enhanced intrinsic excitability. Interestingly, while investigating early α-syn–dependent functional changes in SNpc DA neurons, we demonstrated in a recent study that α-syn aggregates bidirectionally affect the spontaneous firing activity of nigral DA neurons in a time-dependent manner.[15] Specifically, intrastriatal injection of α-syn–preformed fibrils (PFF-α-syn), which retrogradely accumulate into nigral DA neurons, caused early inhibition of spontaneous firing while, at a later time-point, it increased firing frequency and enhanced excitability.[15] A similar biphasic effect of progressive α-syn overload is now overt in the *Snca*$^{+/+}$ rat model. Actually, although we previously reported that SNpc DA neurons from 5-month-old *Snca*$^{+/+}$ rats displayed a reduced firing rate with respect to control DA neurons,[23] our present data from 12- to 13-month-old *Snca*$^{+/+}$ rats demonstrate increased DA neurons' excitability. This posits the switch from early inhibition toward late hyperexcitability of nigral DA neurons as a pathological mechanism of progressive α-syn overload. According to this hypothesis, a reduction of the spontaneous firing rate of SNpc DA neurons has been reported in another *Snca* overexpressing mouse model in the absence of α-syn aggregates,[20] possibly indicating that such electrophysiological alteration occurs in nigral DA neurons before or during the initial α-syn aggregation. Moreover, early α-syn–dependent inhibition of firing activity of SNpc DA neurons can be induced by acute α-syn injection in single DA neurons via the patch-clamp technique, causing hyperpolarization of membrane potential reliant on ATP-sensitive potassium channels (K_{ATP}) activation.[24] Furthermore, increased firing frequency of SNpc DA neurons has also been linked to the overexpression of mutant α-syn in mice (namely, *Snca* harboring the A53T point mutation A53T-*Snca*)[21] through an α-syn–induced oxidative impairment of voltage-activated K^+ channels.[16]

To better outline functional mechanisms by which α-syn overload leads to increased firing and hyperexcitability of nigral DA neurons, we measured various intrinsic conductances that control firing frequency, regularity, and mode of discharge of nigral DA neurons, namely, (1) I_h, mediated by HCN channels; (2) I_{Kv}, mediated by depolarization-gated K^+ channels (K_v); and (3) I_{AHP}, mediated by different K^+ channels subtypes, including SK and BK.[31] We found that I_h in nigral DA neurons is not modified by prolonged α-syn overexpression, with preserved peak currents or current-to-voltage relationships in aged *Snca*$^{+/+}$ similar to WT rats. Of note, although I_h is affected in nigral DA neurons in PD animal models induced by injection of neurotoxins as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or in spontaneously overexpressing α-syn rats,[22,57,58] recent evidence supports that this current is less vulnerable to α-syn overload. Actually, in line with present data, I_h appears conserved in nigral DA neurons also in other α-syn–based PD animal models, encompassing intrastriatal PFF-α-syn injection[15] or A53T-*Snca* overexpression.[21]

Interestingly, prolonged α-syn overload affected K_v activity, as demonstrated by reduced A-type I_{Kv} in nigral DA neurons from aged *Snca*$^{+/+}$ rats compared with WT rats. According to our data, an α-syn–dependent K_v impairment in nigral DA neurons has been previously reported in A53T-*Snca* overexpressing mice,[21] indicating that K_v hypofunction represents a functional alteration triggered by either normal or mutated α-syn overload. Of note, K_v activity shapes action potential waveform, tuning firing rate and excitability of nigral DA neurons,[33,37,38] and it has already been reported that impairment of K_v increases firing activity and excitability of SNpc DA neurons in the A53T-*Snca* overexpressing mice model.[21]

Finally, we found that prolonged α-syn overexpression is associated with reduced I_{AHP}, which relies on a selective BK channel hypofunction. Apamin-sensitive SK channels similarly contributed to I_{AHP} in

nigral DA neurons from *Snca*$^{+/+}$ and WT rats, whereas the IbTX-induced I_{AHP} reduction was occluded in *Snca*$^{+/+}$ DA neurons. This observation demonstrates that a selective impairment of BK channels, rather than SK, accounts for the reduced I_{AHP} observed in the nigral DA neurons of aged *Snca*$^{+/+}$ rats. By affecting the afterhyperpolarization/repolarization phase of action potentials, BK channel hypofunction can enhance spontaneous firing frequency and excitability, determining the higher proportion of active or bursting cells in aged *Snca*$^{+/+}$ rats. Accordingly, it has already been demonstrated that IbTX-induced BK channel inhibition increases the spontaneous firing frequency of SNpc DA neurons.[36] Regarding the impact of I_{AHP} changes in the α-syn–dependent regulation of firing rate, in younger 5-month-old *Snca*$^{+/+}$ rats, an increase in I_{AHP} correlates with inhibition of the spontaneous firing and hypoexcitability of nigral DA neurons.[23] This further supports bidirectional and age-dependent regulation of SNpc DA neurons during progressive α-syn overload, which could be partially mediated by the augmentation or reduction of I_{AHP}.

In summary, we reveal that prolonged α-syn overload affects two K^+ channel-mediated intrinsic currents that can mutually concur to the increase in spontaneous firing rate and hyperexcitability of nigral DA neurons observed in *Snca*$^{+/+}$ rats. K_v– and BK channel hypofunction could rely on reduced channel expression due to lower dendritic arborization and reduced soma size. However, we could not exclude the interplay of other α-syn–dependent direct or indirect modulatory mechanisms limiting K^+ channel function as well as the contribution of additional mechanisms that may increase firing discharge of DA neurons, including a reduced DA neuron self-inhibition via the D2 autoreceptor,[59] due to lower extracellular DA levels in SN of *Snca*$^{+/+}$ rats or an unbalance of the excitatory/inhibitory inputs to DA neurons.[60]

Of note, because firing rate and modality affect DA release in the striatal projection areas (with the bursting mode more efficient in boosting DA levels than the regular mode),[61,62] the functional adjustments that DA neurons embrace in *Snca*$^{+/+}$ rats (which promote their excitability) might represent an attempted compensatory strategy to rebalance striatal DA levels reduced by the progressive neurodegeneration, and this could initially contribute to preserving motor control and cognitive processes. As time goes by, enhanced activity of DA neurons, which is energy demanding, could further contribute to neuronal demise.[63]

Conclusions

Our data demonstrate that prolonged α-syn overexpression shapes the morphology and function of nigral DA neurons by revealing that during emergent α-syn–dependent neurodegeneration (overt with mild neuronal loss, reduced dendritic arborization and neuronal soma size, and decreased basal DA levels in the SN), residual nigral DA neurons display increased firing activity, switch in firing mode, and hyperexcitability, feasibly reliant on α-syn–dependent impairment of K_v and BK channels. Such functional modifications could be compensatory adjustments adopted by nigral DA neurons in the context of an initial degeneration to maintain a sufficient amount of extracellular DA to permit motor and cognitive functions during PD progression. ■

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Data

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