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# Mechanisms underlying altered striatal synaptic plasticity in old A53T-α synuclein overexpressing mice

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**Abstract**

The interactions between certain α-synuclein (SNCA) conformations and dopamine (DA) metabolism cause selective DA neuron degeneration in Parkinson's disease (PD). Preclinical research on PD took advantage of increasing studies involving different animal models which express different forms of mutated SNCA. Transgenic animals expressing mutant α-synucleins such as mice transgenic for A53T-SNCA (TG) are considered valuable models to assess specific aspects of the pathogenesis of synucleinopathies and PD. In this study we performed electrophysiological recordings in corticostriatal slice preparations from young TG overexpressing mice, in which extracellular striatal DA levels appeared to be normal, and in old TG mice, characterized by abnormalities in striatal DA signaling and impaired long-term depression (LTD). We report no difference in TG mice from the two groups of age of either the basal membrane properties and synaptic striatal excitability in respect to age-matched wild-type mice. Furthermore, in old TG mice, showing plastic abnormalities and motor symptoms, we investigated the mechanisms at the basis of the altered LTD. In old TG mice LTD could not be restored by treatments with acute application of DA or by subchronic treatment with L-3,4-dihydroxyphenylalanine (L-DOPA). Conversely, the application of the phosphodiesterase inhibitor zaprinast fully restored LTD to normal conditions via the stimulation of a cyclic guanosine monophosphate (GMP)-protein kinase G-dependent intracellular signaling pathway. These results suggest that, in addition to the dopaminergic alterations reported in this genetic model of PD, other signal transduction pathways linked to striatal synaptic plasticity are altered in an age-dependent manner.


*Keywords:* Alpha-synuclein; A53T-SNCA mutation; Parkinson's disease; Long-term depression; Dopamine; L-DOPA; Phosphodiesterase; PKG

## 1. Introduction

Alpha-synuclein (SNCA), a protein largely expressed in the central nervous system is the major component of Lewy bodies and the hallmark of synucleinopathies. In Parkinson's disease (PD), neuronal death and Lewy bodies formation are mostly restricted to the substantia nigra pars compacta (Lang and Lozano, 1998). This might be explained by the evidence that interactions between certain SNCA conformations and dopamine (DA) metabolism cause selective DA neuron degeneration in PD (Maries et al., 2003). SNCA missense mutations, such as A53T, as well as increased expression of wild-type (WT) SNCA result in rare forms of PD characterized by early onset and autosomal dominant inheritance (Polymeropoulos et al., 1997; Singleton et al., 2003). The correlation between Lewy body formation and neurodegeneration suggests that the aggregation of SNCA is an important event during disease pathogenesis. Transgenic animals expressing mutant α-synucleins such as mice transgenic for A53T-SNCA expressed using the mouse prion-related protein promoter may be valuable models to assess

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specific aspects of the pathogenesis of synucleinopathies. Despite early redistribution of the mutant overexpressed alpha-synuclein from the presynaptic to the somatodendritic compartment, these mice have no motor pathology and no SNCA accumulations at 6 months of age. After this age, however, they show A53T-alpha-synuclein insolubility and progressive motor impairments (Lotharius et al., 2002; Maries et al., 2003). In the striatum of aged A53T-SNCA overexpressing mice, extracellular dopaminergic signaling is disturbed, paralleled by a depressed postsynaptic DA metabolism and loss of long-term depression (LTD) of synaptic plasticity (Kurz et al., 2010).

The first goal of the present work was to characterize the basal striatal excitability and synaptic transmission in young adult A53T-SNCA mice, before the appearance of detectable SNCA insolubility and occurrence of a behavioral phenotype, as well as in old A53T-SNCA mice showing a clear molecular and motor phenotype. As a second major target of our study, in old A53T-SNCA mice we explored the possible mechanisms at the basis of the impaired striatal synaptic plasticity and focused on possible strategies to restore normal striatal synaptic function (LTD).

## 2. Methods

### 2.1. Animal handling and slice preparation

All the experiments were conducted in conformity with the European Communities Council Directive of November 1986 (86/609/ECC) and in accordance with a protocol approved by the Animal Care and Use Committee at the University of Perugia and IRCCS Fondazione, Santa Lucia, Rome, Italy.

Transgenic mice overexpressing A53T-SNCA under the control of prion-protein promoter (PrPmtA) in an FVB/N background were prepared as previously described (Gispert et al., 2003). Homozygous transgenic (TG) mice were compared with FVB/N mice from the same colony, bred and aged in parallel and of the same sex as WT controls. Two groups of different age were analyzed for each genotype; 4-month-old animals represented adult animals prior to the development of motor impairment (young mice) while 20-month-old mice represented the early stage of clinically manifest disease (old mice).

Corticostriatal coronal slices (thickness, 270 $\mu$m) were cut from young and old WT and TG male mice (10 mice per group) using a vibratome. Preparation and maintenance of corticostriatal slices have been previously described (Calabresi et al., 1992; Picconi et al., 2003, 2011). A single slice was transferred to a recording chamber and submerged in a continuously-flowing Krebs' solution (34 °C; 2.5–3 mL/minute) bubbled with a 95% $O_2$-5% $CO_2$ gas mixture. The composition of the solution was: 126 mM NaCl, 2.5 mM KCl, 1.2 mM $MgCl_2$, 1.2 mM $NaH_2PO_4$, 2.4 mM $CaCl_2$, 10 mM glucose, and 25 mM $NaHCO_3$.

### 2.2. Electrophysiology

Intracellular recordings of striatal medium spiny neurons (MSNs) were obtained by using sharp microelectrodes, pulled from borosilicate glass pipettes, backfilled with 2 M KCl (30–60 MΩ). An Axoclamp 2B amplifier (Molecular Devices, Foster City, CA, USA) was connected in parallel to an oscilloscope (Gould, Valley View, OH, USA) to monitor the signal in "bridge" mode and to a PC for acquisition of the traces using pClamp 10 software (Molecular Devices). After impalement of the neuron, a small amount of current (5–20 pA) was injected via the recording electrode, when necessary. Only neurons electrophysiologically identified as spiny neurons were considered (Calabresi et al., 1992, 1999; Picconi et al., 2011). For extracellular recordings an Axoclamp 2B amplifier (Molecular Devices) was used and recording electrodes, filled with 2 M NaCl (15–20 MΩ), were invariably placed within the striatum close to the cortex. A corticostriatal field potential (FP) was evoked every 10 seconds by means of a bipolar electrode connected to a stimulator unit (Grass Telefactor, West Warwick, RI, USA). The stimulating electrode was located in the white matter between the cortex and the striatum to activate corticostriatal fibers. For the input-output curve, a stimulus of increasing voltage intensity and constant duration (200 $\mu$s) was delivered every 10 seconds prior to the onset of each experiment. Quantitative data are expressed as a percentage of the FP amplitudes in respect to the relative control amplitude values, the latter representing the mean of responses recorded during a stable period. For induction of LTD, a conditioning high frequency stimulation (HFS) protocol of 3 trains (3-second duration at 20-second intervals) was delivered at 100 Hz frequency. Only one experiment involving a conditioning HFS protocol was conducted on a single slice.

### 2.3. Statistical analysis

Offline statistical analysis was performed using Clampfit 10 (Molecular Devices) and GraphPad Prism 3.02 (GraphPad Software, San Diego, CA, USA) software. Two-way analysis of variance (ANOVA) was used for statistical analysis. Values given in the figures and text are mean ± standard error (SE). Significant differences were highlighted with asterisks (*** $p < 0.001$).

### 2.4. Drugs

Dopamine hydrochloride, L-3,4-dihydroxyphenylalanine (L-DOPA), benserazide, and zaprinast were purchased from Sigma-Aldrich (Milan, Italy) whereas Rp-8-Br-cGMPS was from Merck Chemicals (Nottingham, UK). Subchronic treatment with L-DOPA was performed in mice by administration of 20 mg/kg L-DOPA plus 7.5 mg/kg benserazide (intraperitoneally) once a day for 4 consecutive days.

## 3. Results

### 3.1. Effect of A53T-SNCA mutation on intrinsic striatal excitability in young and old mice

In order to evaluate whether A53T-SNCA mutation produced age-dependent changes of synaptic transmission, we first measured basal membrane properties of MSNs and performed single-cell recordings in corticostriatal slices obtained from TG and WT mice at both 4 (young mice) and 20 (old mice) months of age. Only neurons electrophysiologically identified as spiny neurons were considered (Calabresi et al., 1992; Picconi et al., 2011). As presented in Figs. 1A and 2A, membrane properties of MSNs in TG and WT mice were similar either in young mice (Fig. 1Aa and b) and in old mice (Fig. 2Ba and b). All these neurons were silent at rest either in young ($V_{rest}$ = −83.8 ± 3 mV, $n$ = 8 in WT and −80.2 ± 4 mV, $n$ = 8 in TG) and in old mice ($V_{rest}$ = −82.2 ± 5 mV, $n$ = 8 in WT and −84.8 ± 7 mV, $n$ = 7 in TG); they displayed membrane rectification and presented a similar tonic firing discharge after injection of positive superthreshold amount of current (Figs. 1Aa, b and 2Aa, b). Furthermore, excitability of striatal neurons was also evaluated evoking corticostriatal FP in young and old mice of both TG and WT group by stimulating glutamatergic afferents to the striatum. Increasing stimulation levels (voltage, 20–45 V; duration, 200 μs) of the corticostriatal pathway evoked FP of similar increasing amplitude in TG and WT mice of both the considered ages, as shown in the plots of the input-output curve (Figs. 1Ac and 2Ac). All together, these data show no significant age-related differences in striatal excitability in A53T-SNCA overexpressing mice.

### 3.2. Age-dependent corticostriatal long-term depression in A53T-SNCA overexpressing mice

Altered corticostriatal long-term depression (LTD) of synaptic plasticity has been previously described in these A53T-SNCA overexpressing mice at old age, in conjunction with increased striatal DA content, but a postsynaptic depression of DA response (Kurz et al., 2010). Here we applied a high-frequency stimulation protocol (HFS) to compare LTD in young and old mice from the TG and WT group. After recording an FP of a stable amplitude for 15–20 minutes, HFS was delivered (Calabresi et al., 1992) and the FP amplitude was monitored for 40 subsequent minutes. As presented in Figs. 1B and 2B, recordings from young mice showed normal LTD either in TG (20.4 ± 4.6%; $n$ = 7) and in WT mice (22.0 ± 3.9%; $n$ = 7; Fig. 1B), while recordings from old animals showed normal LTD only in WT mice (15.4 ± 1.7%; $n$ = 9) but absent LTD in TG mice (TG, $n$ = 6 vs. WT, $n$ = 9; $F(1,143)$ = 82.2; $p < 0.001$) (Fig. 2B).

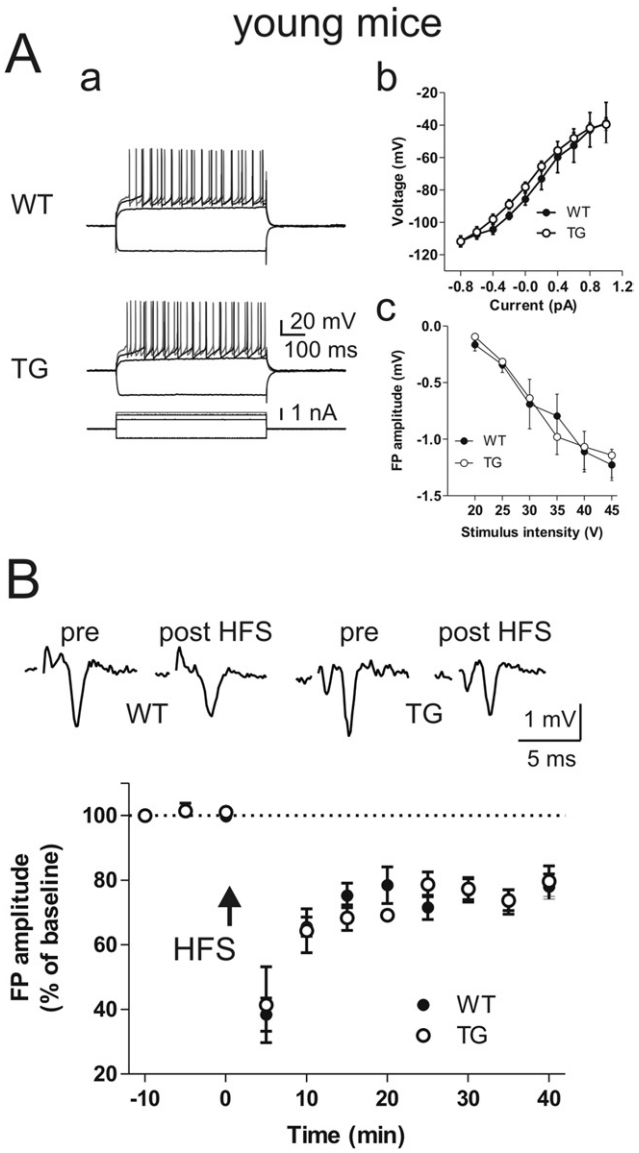


Fig. 1. Intrinsic striatal excitability and synaptic plasticity in young A53T-α-synuclein (SNCA) mice. (A) Intracellular recordings of striatal medium spiny neurons (MSNs) from a young wild-type (WT; top) and A53T-SNCA mouse (TG; bottom) showing the voltage response to hyperpolarizing and depolarizing steps of current (a). Graphs showing the current-voltage plots (b) of several MSNs and the field potentials (FPs) input-output curves (c) acquired in striatal slices from young WT and TG mice. (B) The time course of the field potential (FP) amplitude after the delivery of high-frequency stimulation (HFS) recorded from slices of young WT and TG mice show a normal long-term depression (LTD) of synaptic plasticity in both WT and TG mice of this age. Upper traces show examples of FPs recorded before and 40 minutes post HFS in WT and TG mice. Note that a normal LTD is obtained in both the groups of these young mice.

### 3.3. Dopamine does not restore long-term depression in old A53T-SNCA mice

Increased striatal DA levels in old TG mice might represent either a compensatory mechanism to enhance the dopaminergic signaling or an abnormality in the release of

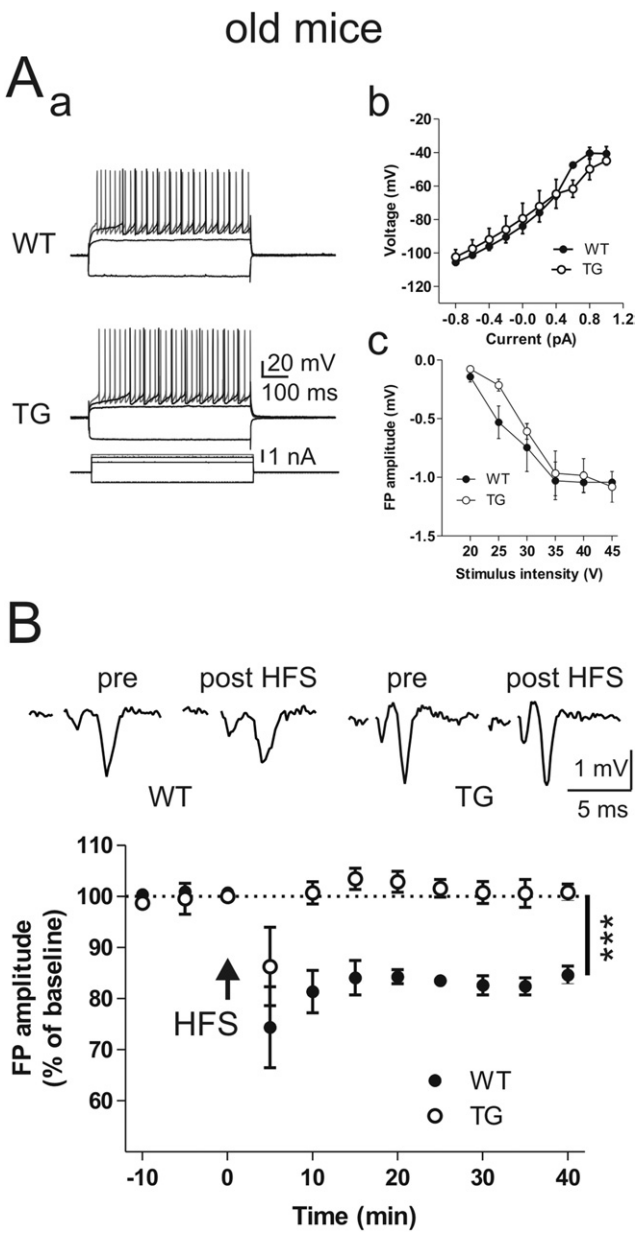


Fig. 2. Intrinsic striatal excitability and synaptic plasticity in old A53T-α-synuclein (SNCA) mice. (A) Intracellular recordings of medium spiny neurons (MSNs) from an old wild-type (WT; top) and old transgenic (TG) mouse (bottom) showing the voltage response to hyperpolarizing and depolarizing steps of current (a). Graphs of current-voltage plots (b) of several MSNs and the field potential (FP) input-output curves (c) measured in striatal slices from old WT and TG mice. (B) The time course of striatal FPs show a normal long-term depression (LTD) only in slices from old WT but not from old TG mice, where LTD is lost (*** $p < 0.001$). Examples of FPs (upper traces) recorded before the delivery of the high-frequency stimulation (HFS) protocol and 40 minutes after the tetanus show a normal LTD of synaptic transmission in old WT animals (left) but no LTD in old TG mice.

DA (Kurz et al., 2010). The concomitant absence of striatal long-term depression of synaptic plasticity might in turn express the physiological outcome of this striatal DA unbalance. In the attempt to restore striatal function in old TG mice, we recorded FP in the presence of 30 μM DA bath applied for the duration of the experiment. Under these conditions a tetanic stimulation produced only a transient reduction of FP amplitude (within 20 minutes from the application of HFS protocol) but it returned to pre-HFS values after 30–40 minutes from HFS application ($n = 8$; Fig. 3A). Because acute administration of DA failed to restore LTD, we also tried to restore a normal LTD in these animals with a subchronic treatment with L-DOPA (20 mg/kg) for 4 consecutive days (see 2. Methods). Also in this case FP recordings from slices from these mice showed a reduction of FP amplitude lasting 20 minutes from HFS protocol returning to pre-HFS conditions within 40 minutes from HFS delivery ($n = 9$; Fig. 3B). Taken together these experiments rule out the possibility that, in our model, exogenous application of dopaminergic agents over days is capable to restore physiological levels of LTD in old A53T-SNCA TG mice.

### 3.4. Striatal long-term depression is restored in old A53T-SNCA mice by enhancing cGMP-PKG-dependent intracellular signaling

In old A53T-SNCA mice multiple signal transduction mechanisms are altered including phosphodiesterases (Kurz et al., 2010). Moreover, we have previously shown that the nitric oxide (NO)/cyclic GMP (cGMP)/protein kinase G (PKG) pathway plays a critical role in the expression of physiological striatal LTD (Calabresi et al., 1999). More recently we have found that zaprinast, an inhibitor of phosphodiesterases (PDEs), is able to rescue LTD by targeting this pathway in a neurotoxic model of PD (Picconi et al., 2011). For these reasons, we decided to test whether the impaired LTD in old TG mice could be restored by this inhibitor of PDEs. We therefore recorded corticostriatal FPs from old TG mice in the continuous presence of 1 μM zaprinast. In these conditions the delivery of HFS protocol produced a significant long-lasting depression of FPs. In fact, FP amplitude was reduced 14.9 ± 3.8% 40 minutes after HFS (TG in zaprinast, $n = 8$ vs. TG, $n = 6$; $F(1,131) = 41.6$; $p < 0.001$; Fig. 4). We studied the role of cGMP/PKG in therapeutic effect of zaprinast by measuring striatal synaptic plasticity in the continuous presence of 1 μM zaprinast coapplied together with 1 μM RP-8Br-cGMPS, a compound known to block PKG function. Interestingly, in the presence of the PKG inhibitor the restorative effect of zaprinast on LTD was blocked (99.0 ± 3.3%; $n = 6$; Fig. 4).

## 4. Discussion

### 4.1. Major findings

In the present work we studied striatal function in physiological conditions and in the parkinsonian state by utilizing the transgenic mice model of PD that overexpresses the human SNCA protein with the A53T mutation. We obtained

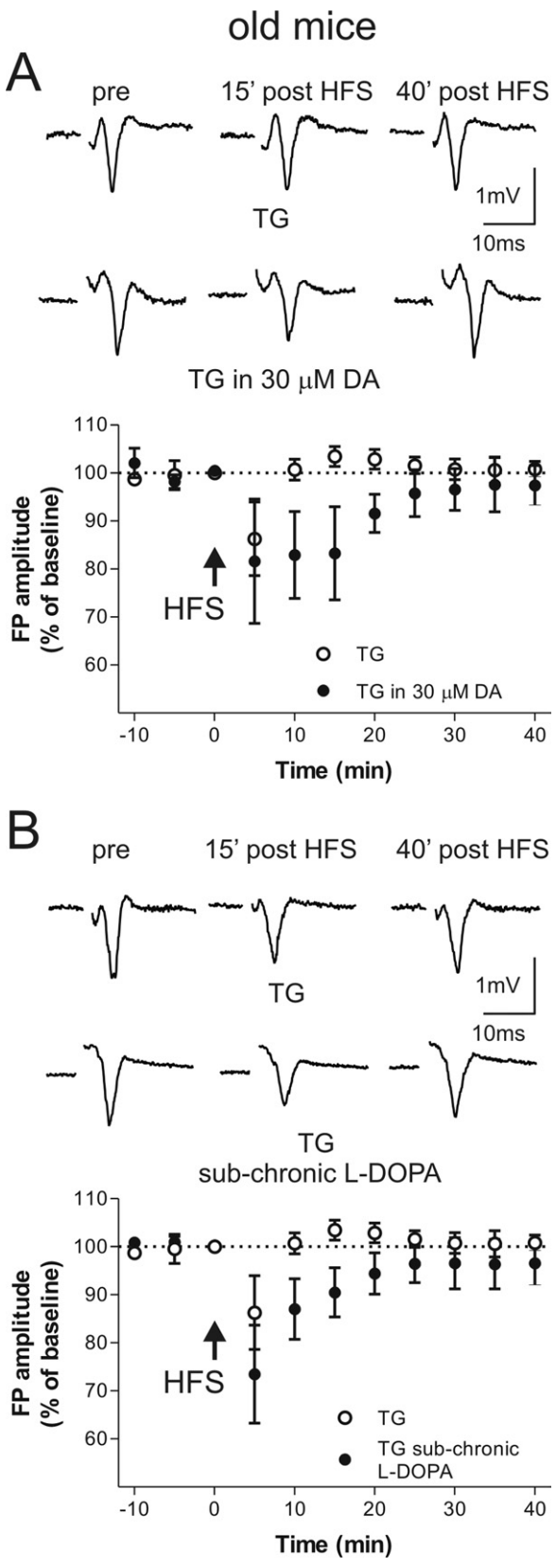


Fig. 3. Acute application of dopamine (DA) or subchronic treatment with L-DOPA do not restore a normal striatal long-term depression (LTD) in old A53T-α-synuclein (SNCA) mice. (A) Time course of the striatal field potential (FP) amplitude before the high-frequency stimulation (HFS) induction and for 40 minutes after the tetanus shows no LTD in old transgenic (TG) mice recorded either in the presence of 30 μM DA bath-applied for the duration of the experiment or in the absence of this drug. Upper traces shows an example of FP recordings acquired before the HFS protocol, 15 and 40 minutes after the tetanus in slices from TG mice recorded in the presence of 30 μM DA and without application of this drug. Note the presence of a short-term depression within 20 minutes from the delivery of HFS protocol. (B) Time course shows the absence of LTD in old TG mice subchronically treated with L-DOPA. Traces show an example of FPs recorded before the HFS induction, 15 minutes and 40 minutes after the tetanus in a slice from an L-DOPA-treated old TG mouse in comparison with a non-treated old TG animal. Also in this case note the presence of a short-term depression within 20 minutes from the delivery of HFS protocol.

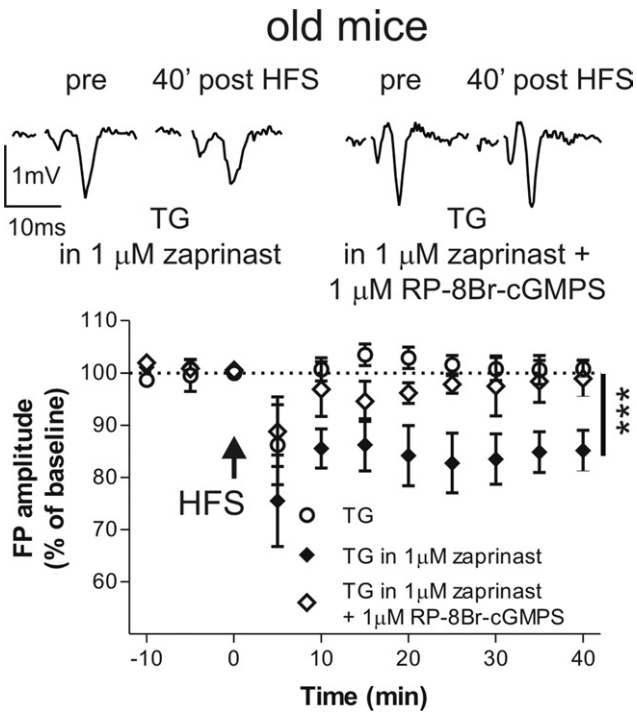


Fig. 4. The phosphodiesterase inhibitor zaprinast restores normal striatal long-term depression (LTD) in old A53T-α-synuclein (SNCA) mice via a protein kinase G (PKG)-dependent mechanism. The time course of field potential (FP) amplitude measured in old TG mice in the presence of 1 μM of the phosphodiesterase inhibitor zaprinast shows a full recovery of striatal LTD (*** $p < 0.001$). Conversely, in the presence of 1 μM zaprinast plus 1 μM of the PKG inhibitor RP-8Br-cGMPS, LTD could not be restored. Upper traces show FPs recorded from an old TG mouse either in the presence of 1 μM zaprinast (left) or in the presence of 1 μM zaprinast plus 1 μM of the PKG inhibitor RP-8Br-cGMPS (right) before the high-frequency stimulation (HFS) and 40 minutes after the tetanus.

3 new major findings having both physiological and clinical relevance.

First we measured the striatal excitability and long-term synaptic plasticity in this animal model of PD at two different stages of the development of the disease. Mice of 4 months of age did not present any pathologic alterations while old mice

display features of a pathologic phenotype. Interestingly, we found that while in young and old animals the basal electrical properties were similar and were not different from aged-matched WT mice, only young TG mice presented a normal LTD whereas in old TG animals LTD was absent.

The second aim of this study was the investigation of the possible mechanisms that might be involved in the altered LTD in these old TG mice. Because striatal DA levels are known to be primarily important in sustaining a normal striatal function (Calabresi et al., 1992, 2007) and an altered striatal DA metabolism has been recently described in these old TG mice (Kurz et al., 2010), we boosted DA receptor activity in the attempt to restore DA signaling and LTD in these old animals. However, we found that neither acute application of DA nor a subchronic treatment with L-DOPA was able to restore a normal LTD in this animal model of PD.

As the third major issue of the present work, we found that pharmacological inhibition of the intracellular phosphodiesterases by zaprinast was able to restore a normal LTD in old TG mice indicating that in this animal model of PD the modulation of the cGMP/PKG intracellular pathway represents an important subject for a rescue of a physiological striatal LTD and a possible target for a new experimental therapeutic approach. In agreement with this hypothesis, the inhibition of PKG was able to suppress the therapeutic effect of zaprinast.

### 4.2. A53T-SNCA mouse model of PD

Alpha-synuclein is a highly conserved 140 amino acid protein whose native function is still being investigated. Among the several transgenic animal models expressing mutant $\alpha$-synucleins, generated to study specific aspects of the pathogenesis of synucleinopathies, we have used mice carrying the human A53T-SNCA transgene, a model that reproduces some of the features of idiopathic PD. In these transgenic mice the overexpression and mutation enhance pathological localization and insolubility of A53T-SNCA, sufficient to produce a progressive neuronal dysfunction in the absence of demonstrable inclusion bodies or of protein aggregations, neuronal loss within the dopaminergic projection or motor pathology until 18–24 months of age (Gispert et al., 2003). Accordingly, we found in these TG mice an age-dependent alteration of the striatal function; in fact, while young TG mice presented normal basal electrophysiological properties and a physiological long-term depression of synaptic transmission, old TG mice presented no LTD, confirming a previous study obtained in A53T-SNCA mice of similar age (Kurz et al., 2010).

### 4.3. Involvement of DA and cGMP/PKG signaling pathways

Explanation for the observed lack of striatal LTD found in old TG mice might imply the impaired DA neurotransmitter release (Kurz et al., 2010). Recent reports pointed to several possible functional interactions between SNCA protein, which is abundantly expressed in presynaptic nerve terminals, and the DA neurotransmitter system including regulation of synaptic DA vesicles, release of DA, modulation of DA synthesis and transport to the plasma membrane (Sidhu et al., 2004; Venda et al., 2010). Abnormal levels of striatal DA content have been described in several in vivo and in vitro animal models of PD such as the hemiparkinsonian rat model of PD obtained with unilateral injection of 6-hydroxydopamine into the nigrostriatal projections, in which striatal DA is strongly reduced (Picconi et al., 2004, 2011), or transgenic mouse models of PD overexpressing truncated fragments of human SNCA, in which a decline in striatal DA levels is documented with increasing age together with a parallel age-related reduction in spontaneous locomotion (Tofaris et al., 2006; Wakamatsu et al., 2008). Similarly, in the striatum of old A53T-SNCA mice, a progressive reduction in the postsynaptic DA response correlated with the absence of LTD and the reduced spontaneous locomotor activity in open-field tests (Kurz et al., 2010). Interestingly, while treatments with DA agonists or with L-DOPA could completely restore the electrophysiological-impaired LTD (Calabresi et al., 2007) and behavioral deficits (Wakamatsu et al., 2008), in some PD animal models, we detected only a short-term depression in old TG mice treated with DA or L-DOPA while these treatments failed to produce a normal long-lasting synaptic depression. These data confirm that a pathological striatal neurotransmission and synaptic plasticity underlie the progressive movement deficit seen in A53T-SNCA mice and suggest that alternative signaling pathways need to be explored in the attempts to rescue a physiological LTD in these old TG mice.

Long-lasting changes in the efficacy of excitatory neurotransmission in the striatum are triggered by the stimulation of complex cascades of intracellular second messenger systems and formation of LTD requires also the activation of NO/cGMP/PKG enzymatic cascade, a crucial pathway subserved by a specific intrastriatal circuitry of nitric oxide synthase (NOS) positive interneurons innervating striatal projecting spiny neurons (Calabresi et al., 1999; Centonze et al., 1999, 2003). Furthermore, the NO signaling pathway can be considered as a possible regulatory mechanism involved in neurodegeneration of dopaminergic fibers in the striatum (Chalimoniuk et al., 2004); in fact altered forms of SNCA producing overstimulation of NOS activity can cause neuronal death by increasing oxidative stress and impairing mitochondria function (Adamczyk et al., 2009). It was found that the in vivo administration of NO donor drugs increased the extracellular level of DA in the striatum (Trabace and Kendrick, 2000). The importance of maintaining a physiological NOS activity is also suggested in the work by Choi et al. (2009) in which, in an in vitro model of PD, mitochondrial injury, and death of striatal neurons were prevented by treatment with a NOS inhibitor (Choi et al., 2009).

NOS positive striatal interneurons are therefore a possible sensitive target of modified forms of SNCA and in the

striatum of our old A53T-SNCA mice their sufferance or their altered metabolism might be responsible for the absence of LTD.

In the present work we verified this hypothesis by stimulating intracellular striatal NO/cGMP/PKG by inhibiting PDEs. Although central neurons express various families of PDEs (Beavo, 1995), striatal neurons are highly enriched with calmodulin-dependent PDE (Polli and Kincaid, 1994; Yan et al., 1994). For this reason we used the specific cGMP-PDEs inhibitor zaprinast (Calabresi et al., 1999) and interestingly this treatment fully restored striatal LTD in all the old A53T-SNCA mice tested. Zaprinast specifically targets PDE5, 6, and 9 and even if striatal neurons display low PDE5 levels, zaprinast increased cGMP striatal levels and affected striatal behavior in animal models suggesting that this drug may still be a useful tool to study NO/cGMP/PKG pathway within the nucleus striatum (Domek-Łopacińska and Strosznajder, 2008; Giorgi et al., 2008; Puerta et al., 2009). In addition, we confirmed the important effect of zaprinast in our mice model by blocking PKG function in old TG mice in the presence of this PDEs inhibitor. Because formation of LTD requires the activation of PKG, even boosting cGMP levels by inhibition of PDEs was not effective in restoring LTD if the function of this PKG was preventively blocked. The possibility to modulate striatal LTD by affecting NO/cGMP/PKG pathway in old A53T-SNCA mice may raise the interest in correlating striatal synaptic plasticity and possible alterations of the number and/or the metabolism of NOS positive interneurons in these mouse models of PD. Interestingly, the presence of human transgenic A53T-alpha-synuclein have not been detected in the cell body of striatal neurons of old A53T-SNCA mice, revealing its presence only located in the synaptic cell terminals (Gispert et al., 2003). However, an age-dependent morphological analysis of NOS positive striatal interneurons will require further attention. Moreover, further studies will be required to investigate the possibility that systemic administration of PDE inhibitors ameliorate motor performances altered in old A53T-SNCA mice, in line with our in vitro data showing that zaprinast restores LTD in these mice.

Taken together these findings endorse the possibility that the modulation of NO/cGMP/PKG pathway might be a possible additional strategy to gain insights into the mechanisms occurring in the A53T-SNCA mouse model of PD.

## Disclosure statement

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All authors disclose no conflicts of interest.

All the experiments were conducted in conformity with the European Communities Council Directive of November 1986 (86/609/ECC) and in accordance with a protocol approved by the Animal Care and Use Committee at the University of Perugia and IRCCS Fondazione, Santa Lucia, Rome, Italy.

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## References

Adamczyk, A., Czapski, G.A., Kaźmierczak, A., Strosznajder, J.B., 2009. Effect of *N*-methyl-D-aspartate (NMDA) receptor antagonists on alpha-synuclein-evoked neuronal nitric oxide synthase activation in the rat brain. Pharmacol. Rep. 61, 1078–1085.

Beavo, J.A., 1995. Cyclic nucleotide phosphodiesterases: functional implications of multiple isoforms. Physiol. Rev. 75, 725–748.

Calabresi, P., Maj, R., Pisani, A., Mercuri, N.B., Bernardi, G., 1992. Long-term synaptic depression in the striatum: physiological and pharmacological characterization. J. Neurosci. 12, 4224–4233.

Calabresi, P., Gubellini, P., Centonze, D., Sancesario, G., Morello, M., Giorgi, M., Pisani, A., Bernardi, G., 1999. A critical role of the nitric oxide/cGMP pathway in corticostriatal long-term depression. J. Neurosci. 19, 2489–2499.

Calabresi, P., Picconi, B., Tozzi, A., Di Filippo, M., 2007. Dopamine-mediated regulation of corticostriatal synaptic plasticity. Trends Neurosci. 30, 211–219.

Centonze, D., Gubellini, P., Bernardi, G., Calabresi, P., 1999. Permissive role of interneurons in corticostriatal synaptic plasticity. Brain Res. Brain Res. Rev. 31, 1–5.

Centonze, D., Gubellini, P., Pisani, A., Bernardi, G., Calabresi, P., 2003. Dopamine, acetylcholine and nitric oxide systems interact to induce corticostriatal synaptic plasticity. Rev. Neurosci. 14, 207–216.

Chalimoniuk, M., Langfort, J., Lukacova, N., Marsala, J., 2004. Upregulation of guanylyl cyclase expression and activity in striatum of MPTP-induced parkinsonism in mice. Biochem. Biophys. Res. Commun. 324, 118–126.

Choi, D.Y., Liu, M., Hunter, R.L., Cass, W.A., Pandya, J.D., Sullivan, P.G., Shin, E.J., Kim, H.C., Gash, D.M., Bing, G., 2009. Striatal neuroinflammation promotes Parkinsonism in rats. PLoS One 4, e5482.

Domek-Łopacińska, K., Strosznajder, J.B., 2008. The effect of selective inhibition of cyclic GMP hydrolyzing phosphodiesterases 2 and 5 on learning and memory processes and nitric oxide synthase activity in brain during aging. Brain Res. 1216, 68–77.

Giorgi, M., D'Angelo, V., Esposito, Z., Nuccetelli, V., Sorge, R., Martorana, A., Stefani, A., Bernardi, G., Sancesario, G., 2008. Lowered cAMP and cGMP signalling in the brain during levodopa-induced

dyskinesias in hemiparkinsonian rats: new aspects in the pathogenetic mechanisms. Eur. J. Neurosci. 28, 941–950.

Gispert, S., Del Turco, D., Garrett, L., Chen, A., Bernard, D.J., Hamm-Clement, J., Korf, H.W., Deller, T., Braak, H., Auburger, G., Nussbaum, R.L., 2003. Transgenic mice expressing mutant A53T human alpha-synuclein show neuronal dysfunction in the absence of aggregate formation. Mol. Cell. Neurosci. 24, 419–429.

Kurz, A., Double, K.L., Lastres-Becker, I., Tozzi, A., Tantucci, M., Bockhart, V., Bonin, M., García-Arencibia, M., Nuber, S., Schlaudraff, F., Liss, B., Fernández-Ruiz, J., Gerlach, M., Wüllner, U., Lüddens, H., Calabresi, P., Auburger, G., Gispert, S., 2010. A53T-alpha-synuclein overexpression impairs dopamine signaling and striatal synaptic plasticity in old mice. PLoS One 5, e11464.

Lang, A.E., Lozano, A.M., 1998. Parkinson's disease. First of two parts. N Engl. J. Med. 339, 1044–1053.

Lotharius, J., Barg, S., Wiekop, P., Lundberg, C., Raymon, H.K., Brundin, P., 2002. Effect of mutant alpha-synuclein on dopamine homeostasis in a new human mesencephalic cell line. J. Biol. Chem. 277, 38884–38894.

Maries, E., Dass, B., Collier, T.J., Kordower, J.H., Steece-Collier, K., 2003. The role of alpha-synuclein in Parkinson's disease: insights from animal models. Nat. Rev. Neurosci. 4, 727–738.

Picconi, B., Bagetta, V., Ghiglieri, V., Paillè, V., Di Filippo, M., Pendolino, V., Tozzi, A., Giampà, C., Fusco, F.R., Sgobio, C., Calabresi, P., 2011. Inhibition of phosphodiesterases rescues striatal long-term depression and reduces levodopa-induced dyskinesia. Brain 134, 375–387.

Picconi, B., Centonze, D., Håkansson, K., Bernardi, G., Greengard, P., Fisone, G., Cenci, M.A., Calabresi, P., 2003. Loss of bidirectional striatal synaptic plasticity in L-DOPA-induced dyskinesia. Nat. Neurosci. 6, 501–506.

Picconi, B., Centonze, D., Rossi, S., Bernardi, G., Calabresi, P., 2004. Therapeutic doses of L-dopa reverse hypersensitivity of corticostriatal D2-dopamine receptors and glutamatergic overactivity in experimental parkinsonism. Brain 127, 1661–1669.

Polli, J.W., Kincaid, R.L., 1994. Expression of a calmodulin-dependent phosphodiesterase isoform (PDE1B1) correlates with brain regions having extensive dopaminergic innervation. J. Neurosci. 14, 1251–1261.

Polymeropoulos, M.H., Lavedan, C., Leroy, E., Ide, S.E., Dehejia, A., Dutra, A., Pike, B., Root, H., Rubenstein, J., Boyer, R., Stenroos, E.S., Chandrasekharappa, S., Athanassiadou, A., Papapetropoulos, T., Johnson, W.G., Lazzarini, A.M., Duvoisin, R.C., Di Iorio, G., Golbe, L.I., Nussbaum, R.L., 1997. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science 276, 2045–2047.

Puerta, E., Hervias, I., Goñi-Allo, B., Lasheras, B., Jordan, J., Aguirre, N., 2009. Phosphodiesterase 5 inhibitors prevent 3,4-methylenedioxymethamphetamine-induced 5-HT deficits in the rat. J. Neurochem. 108, 755–766.

Sidhu, A., Wersinger, C., Vernier, P., 2004. Does alpha-synuclein modulate dopaminergic synaptic content and tone at the synapse? FASEB J. 18, 637–647.

Singleton, A.B., Farrer, M., Johnson, J., Singleton, A., Hague, S., Kachergus, J., Hulihan, M., Peuralinna, T., Dutra, A., Nussbaum, R., Lincoln, S., Crawley, A., Hanson, M., Maraganore, D., Adler, C., Cookson, M.R., Muenter, M., Baptista, M., Miller, D., Blancato, J., Hardy, J., Gwinn-Hardy, K., 2003. alpha-Synuclein locus triplication causes Parkinson's disease. Science 302, 841.

Tofaris, G.K., Garcia Reitböck, P., Humby, T., Lambourne, S.L., O'Connell, M., Ghetti, B., Gossage, H., Emson, P.C., Wilkinson, L.S., Goedert, M., Spillantini, M.G., 2006. Pathological changes in dopaminergic nerve cells of the substantia nigra and olfactory bulb in mice transgenic for truncated human alpha-synuclein(1–120): implications for Lewy body disorders. J. Neurosci. 26, 3942–3950.

Trabace, L., Kendrick, K.M., 2000. Nitric oxide can differentially modulate striatal neurotransmitter concentrations via soluble guanylate cyclase and peroxynitrite formation. J. Neurochem. 75, 1664–1674.

Venda, L.L., Cragg, S.J., Buchman, V.L., Wade-Martins, R., 2010. $\alpha$-Synuclein and dopamine at the crossroads of Parkinson's disease. Trends Neurosci. 33, 559–568.

Wakamatsu, M., Iwata, S., Funakoshi, T., Yoshimoto, M., 2008. Dopamine receptor agonists reverse behavioral abnormalities of alpha-synuclein transgenic mouse, a new model of Parkinson's disease. J. Neurosci. Res. 86, 640–646.

Yan, C., Bentley, J.K., Sonnenburg, W.K., Beavo, J.A., 1994. Differential expression of the 61 kDa and 63 kDa calmodulin-dependent phosphodiesterases in the mouse brain. J. Neurosci. 14, 973–984.