Supplementary Materials and Methods

Experimental animals

Forty-eight 8-week old male Sprague-Dawley rats (approximately 250 g; Envigo, Spain) were used in this study. Animals were housed six per cage under standard laboratory conditions (22 ± 1ºC. 55 ± 5% of humidity and 12:12h light/dark cycle) with ad libitum access to food and water. Experimental procedures complied with the ARRIVE guidelines and were performed in accordance with the European Directive 2010/63/EU and Spanish regulations (RD 53/2013) governing the care and use of laboratory animals. All protocols were reviewed and approved by the Local Committee for Animal Experimentation at the University of the Basque Country (Leioa, Spain; #M20-2021 and #M30-2021-242).

Stereotaxic surgery

To induce α-syn overexpression in the SNpc, stereotaxic surgery was performed by bilateral injection of an AAV vector encoding the human SNCA gene into the SNpc. Animals were anaesthetised with isoflurane (4% for induction and 1-2% for maintenance) and positioned with the head in a horizontal position in a stereotaxic frame (David Kopf® Instruments, U.S.A.). The scalp was incised to expose the skull, and a burr hole (0.5-1mm diameter) was drilled over each injection site using the coordinates of the SNpc (relative to Bregma): AP=5.3, ML=1.8, DV=7.2. A glass capillary connected to a microinjection system was used to deliver the AAV. A volume of 2 µL (1.07×10^{12} vg/mL) was injected at each site at 0.4 µL/min**.** The capillary was left place for 5 min after each injection to allow diffusion of the viral suspension before being carefully removed. After surgery, the incision was sutured and animals received an intraperitoneal injection of saline to promote recovery, and a subcutaneous injection of meloxicam (2 mg/kg) for postoperative analgesia. Following surgery, animals were allocated to two experimental groups: a SHAM group (n = 24), which received bilateral injections of an empty AAV vector (rAAV9-CMVie/SynP-WPRE), and an α-syn group (n = 24), which received bilateral injections of an AAV vector encoding human α-syn (rAAV9-CMVie/SynP-wtsyn-WPRE).

Behavioural evaluation

Behavioural assessment was performed at 1 and 3 month post-injection using a battery of tests to evaluate non-motor and motor behaviour. All tests were performed under a 50-lux ilumination. Animals were acclimatised to the experimental room for 30 min before testing.

Open field

General locomotor activity was assessed using an automated open-field arena (Actitrack®, Panlab-Harvard Apparatus, USA). The apparatus consisted of a 45 × 45 cm square arena equipped with infrared beams spaced 2.5 cm apart and connected to a computerised tracking system, which recorded locomotor activity, distance travelled, rearing behaviour, and time spent in the centre of the arena. Animals were placed in the centre of the arena and allowed to explore freely for 15 min.

Motor behaviour

Motor function was evaluated 1 and 3 months post-injection using the rotarod and bar tests.

Rotarod

Motor coordination and balance were assessed using a Rotarod apparatus (ROTA-ROD/RS, Panlab-Harvard Apparatus, USA). Animals were placed on an accelerating rotating rod (0–40 rpm over 4 min), and latency to fall was recorded. Animals underwent 5 days of preoperative training. On the first day, animals remained on the rotating rod until completion of the training session, whereas during the following four days they completed three trials per day, with latency to fall recorded in each trial. Animals rested for 40 min between consecutive trials. At 1 and 3 months after stereotaxic surgery, animals completed an additional 4-day protocol consisting of 3 training days followed by 1 testing day. During the testing session, each animal performed three trials, and the mean latency to fall was used for statistical analysis.

Bar test

Catalepsy was assessed using the bar test, which measures the ability of animals to correct an externally imposed posture. Animals were placed with both forepaws on a custom-built wooden bar positioned 9 cm above the floor, and the time required to remove both paws and return them to the floor was recorded. Each animal completed three trials, and the mean value was used for analysis.

Non-motor behaviour

Anxiety-like behaviour was evaluated 1 and 3 months post-injection using the elevated plus maze (EPM) and marble burying test (MBT).

Elevated plus maze

The apparatus consisted **of** two open and two closed arms (50 × 10 cm) elevated 73 cm above the floor. The closed arms were enclosed by 33-cm-high walls. At the beginning of each trial, animals were placed in the centre of the maze facing an open arm and allowed to explore freely for 5 min. Behaviour was video-recorded, and the number of entries into the open and closed arms, together with the time spent in the open arms, was quantified.

Marble burying test

The MBT was used to evaluate anxiety-like and compulsive-like behaviour. Animals were placed in Plexiglas cages (45 × 25 × 15 cm) containing 3 cm of bedding, with 20 glass marbles arranged in a 4 × 5 grid. Animals were allowed to explore the cage for 10 min, after which the number of buried marbles was recorded. A marble was considered buried when at least 75% of its surface was covered by bedding.

Histochemical procedures

For histochemical analyses, one hemisphere from each animal was fixed in 4% paraformaldehyde (PFA) and sectioned coronally at 40 μm. Coronal sections were used to confirm α-syn overexpression and assess the extent of dopaminergic neurodegeneration. A complete list of primary antibodies is provided in **Supplementary Table 1.**

Sections containing the striatum and SNpc were immunolabelled for tyrosine hydroxylase (TH) to assess dopaminergic degeneration. Sections were washed three times in PBS, and endogenous peroxidase activity was quenched using 3% H_2O_2 and 10% methanol in PBS for 30 min. Sections were then blocked for 1 h in 5% normal goat serum (NGS) and 0.5% Triton X-100 in PBS, followed by overnight incubation with rabbit anti-TH antibody (1:2000). On the following day, sections were incubated with an avidin–biotin peroxidase complex (ABC kit) for 1 h, and immunoreactivity was visualised using 3,3′-

diaminobenzidine (DAB) and 0.03% H_2O_2. Finally, sections were washed in PBS, mounted onto Superfrost™ slides, dehydrated, and coverslipped using DPX mounting medium**.**

To confirm α-syn expression, the same protocol was followed using a mouse anti-α-syn primary antibody**.** Whole-brain sections were scanned using a high-resolution slide scanner (Pannoramic MIDI II, 3DHISTECH, Hungary).

Double immunofluorescence was performed on sections containing the SNpc and striatum to assess the co-localisation of TH with α-syn and phosphorylated α-syn **(**pSer129-α-syn**),** a marker of early α-syn aggregation**.** Immunofluorescence staining was performed as described above with minor modifications. Images were acquired using a Nikon Ti-U fluorescence microscope equipped with a Ds-Qi2 digital camera (Nikon, Japan) at the SGIker Microscopy Facility (UPV/EHU).

Quantification

Optical density

TH and α-syn immunoreactivity in the striatum was quantified by optical density analysis following DAB staining, as described above. For each animal, serial coronal sections separated by 480 µm were analysed. Optical density measurements were performed using ImageJ/Fiji (National Institutes of Health, USA). For each section, the striatum was manually delineated, and optical density values were corrected for background staining. Background optical density was measured in the cortex, which lacked specific immunoreactivity, and subtracted from each measurement.

Unbiased stereology

The number of TH-positive cells in the SNpc was quantified using unbiased stereology, as previously described. Sampling was performed between -4.56 and -5.64 mm relative to bregma. Cell counting was carried out using the Mercator Image Analysis System (Explora Nova, France) coupled to an Olympus BX51 microscope equipped with a motorised XYZ stage and a digital camera (Olympus, Japan). TH-positive neurons were quantified using the optical fractionator method with a 40× objective. The section sampling fraction (SSF) was 1/6. The SNpc was delineated using a 4× objective, and counting was performed using a 60 × 60 µm counting frame on an 80 × 80 µm sampling grid, with a 5% guard zone. At least eight sections per animal, separated by 240 µm, were analysed according to established stereological counting rules. SNpc volume was estimated using the Cavalieri method.

Membrane isolation

The brain areas of interest were dissected and homogenised using an Ultra-Turrax® T10 basic disperser (IKA, Germany) followed by a Teflon-glass homogenizer (RZR 2020, Heidolph, Germany) in 25 or 50 volumes of homogenisation buffer (1 mM EGTA, 3 mM $MgCl_2$ and 50 mM Tris-HCl, pH 7.4) supplemented with 250 mM sucrose. Homogenates were centrifuged at 3,857 × g for 5 min at 4°C (Microfuge® 22R, Beckman Coulter, USA). The resulting supernatant was collected and centrifuged at **1**8,000 × g for 15 min at 4°C to obtain the membrane fraction. The pellet was washed with homogenisation buffer, centrifuged again under the same conditions, and stored at −80°C until use.

Blood collection

The animals were deeply anesthetised with ketamine (100 mg/mL) and 2% xylazine (80 mg/kg + 10 mg/kg) and the thoracic cavity was opened to collect blood by cardiac puncture from the left ventricle. Blood intended for platelet and PBMC isolation was collected into tubes containing acid-citrate-dextrose (1:9, anticoagulant:blood**)**, whereas blood for serum preparation was collected into tubes without anticoagulant.

Platelets and PBMCs isolation

Platelets and PBMCs were isolated from peripheral blood using Ficoll-Paque™ PREMIUM 1.084 (Cytiva, USA) according to the manufacturer's instructions. Briefly, blood was diluted 1:1 with balanced saline solution (BSS), carefully layered over 3 mL Ficoll, and centrifuged at 400 × g for 40 min (Multifuge 3 S-R, Heraeus, Germany). Following density-gradient centrifugation, the platelet-rich plasma (PRP) and PBMC layer were collected separately.

PRP was centrifuged at 4,562 × g for 15 min to pellet platelets. PBMCs were washed with BSS and centrifuged at 400 × g for 10 min. Cell pellets were stored at −80°C until further analysis.

Serum collection and ELISA multiplex assay

Whole blood was allowed to clot for 2 h at room temperature and centrifuged at 1,500 × g for 15 min. Serum was collected and stored at −80°C until analysis. Inflammatory cytokines (IL-1β, IL-2, IL-6, IL-10, TNF-α and IFN-γ) were quantified using the MILLIPLEX® Rat Cytokine/Chemokine Magnetic Bead Panel (Merck Millipore) according to the manufacturer's instructions. Serum samples were thawed, diluted 1:2 in assay buffer, and analysed following the manufacturer's protocol.

Microarrays fabrication

Cell membrane microarrays (CMMAs) and cell microarrays (CMAs) were fabricated using brain membrane preparations and peripheral blood cells, respectively. Membrane fractions from the cortex (CX), striatum and SNpc were used to generate CMMAs, whereas platelets and PBMCs were used to fabricate CMAs.

Prior to printing, membrane and cell samples were thawed and resuspended in **a** proprietary printing buffer developed by IMG Pharma (IMG Pharma Biotech S.L., Spain) 24 h before microarray fabrication. Samples were loaded into 384-well source plates and printed onto pre-treated glass slides as previously described.36 Microarrays were generated using a non-contact Nano-Plotter NP2.1 microarrayer (GESIM, Germany) equipped with a solenoid dispensing system, under controlled conditions (4°C and 60% relative humidity). Following printing, slides were left under the same environmental conditions for 30 min to allow complete drying before being stored at -20°C until use.

Determination of mitochondrial activity in microarrays

Mitochondrial activity was assessed by measuring the function of individual mitochondrial electron transport chain (mETC) complexes. Superoxide production by NADH dehydrogenase (complex I), succinate dehydrogenase (complex II) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was quantified. In addition, cytochrome C oxidase (CCo, complex IV) activity was determined using 3,3'diaminobenzidine (DAB).

Superoxide production was detected using nitro blue tetrazolium (NBT), as it captures electrons and undergoes a colour change from yellow to blue, thereby facilitating the detection of superoxide. CMMAs and CMAs were incubated with assay solutions containing the appropriate substrate for each mETC complex to determine basal superoxide production. Parallel incubations were performed in the presence of specific mETC inhibitors to determine maximal superoxide production. After incubation at 24°C, microarrays were rinsed with distilled water, scanned, and analysed by optical density. The composition of each assay solution is detailed in **Supplementary Table 2.**

CCO activity was determined using DAB, a reducing agent that donates electrons to oxidised cytochrome C (cytC). Oxidation of DAB generated an insoluble brown reaction product**,** which was quantified by optical density. CMMAs and CMAs were incubated with DAB in order to ascertain their basal activity, and with DAB and cytC to determine their maximum activity at 24°C. They were then rinsed in dH_2O and scanned for further quantification.

Microarray images were analysed using ImageScanner software (IMG Pharma Biotech S.L., Spain). Optical density values were obtained after background subtraction and normalised to the amount of immobilised protein, determined using the Bradford assay. Net enzymatic activity was calculated by subtracting basal from maximal activity, and the resulting values were analysed using GraphPad Prism.

CCO activity was also evaluated kinetically in platelets. These were resuspended in 5 mM phosphate buffer at a final protein concentration of 0.043 mg/mL and incubated in 96-well plates containing 0.5 mg/mL DAB and 0.025% cytochrome c. Absorbance was measured using a Multiskan™ FC microplate reader (Thermo Fisher Scientific, USA) every 5 min for 13 h. Reaction rates were calculated from the linear portion of the kinetic curves and compared between experimental groups. Data analysis was performed using GraphPad Prism.

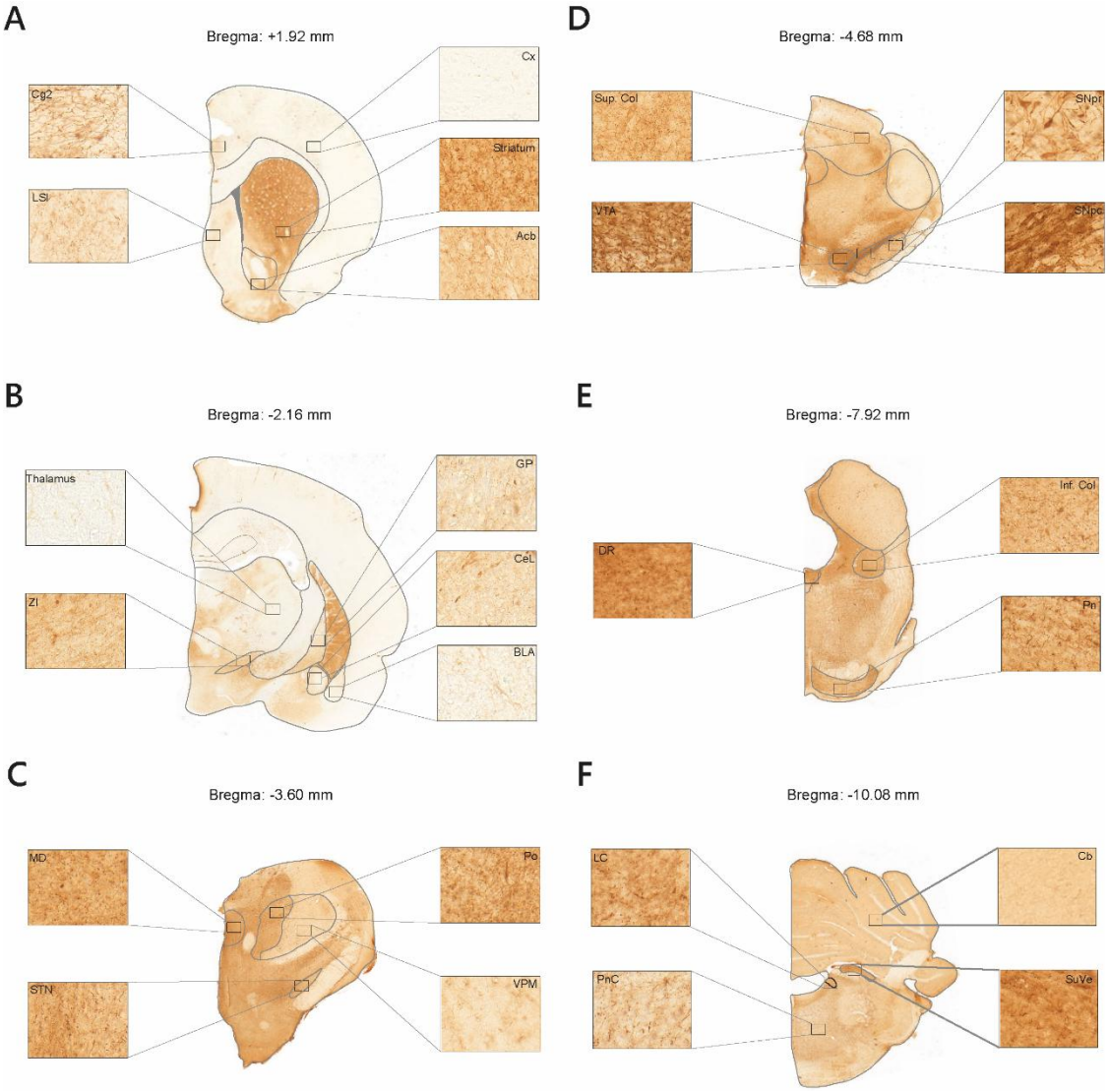
Predictive model

A supervised classification approach was used to evaluate the ability of peripheral mitochondrial biomarkers to discriminate between the SHAM and α-syn groups. Analyses were performed using the Orange data mining software package (University of Ljubljana, Slovenia). Model performance was assessed using five-fold cross-validation**.** Initially, all variables obtained from peripheral blood samples were included in the analysis. Inflammatory cytokines were subsequently excluded because they did not differ significantly between experimental groups. The final models were constructed using the four peripheral variables showing the greatest discriminatory power. Two supervised classification algorithms were evaluated: logistic regression and a neural network classifier.

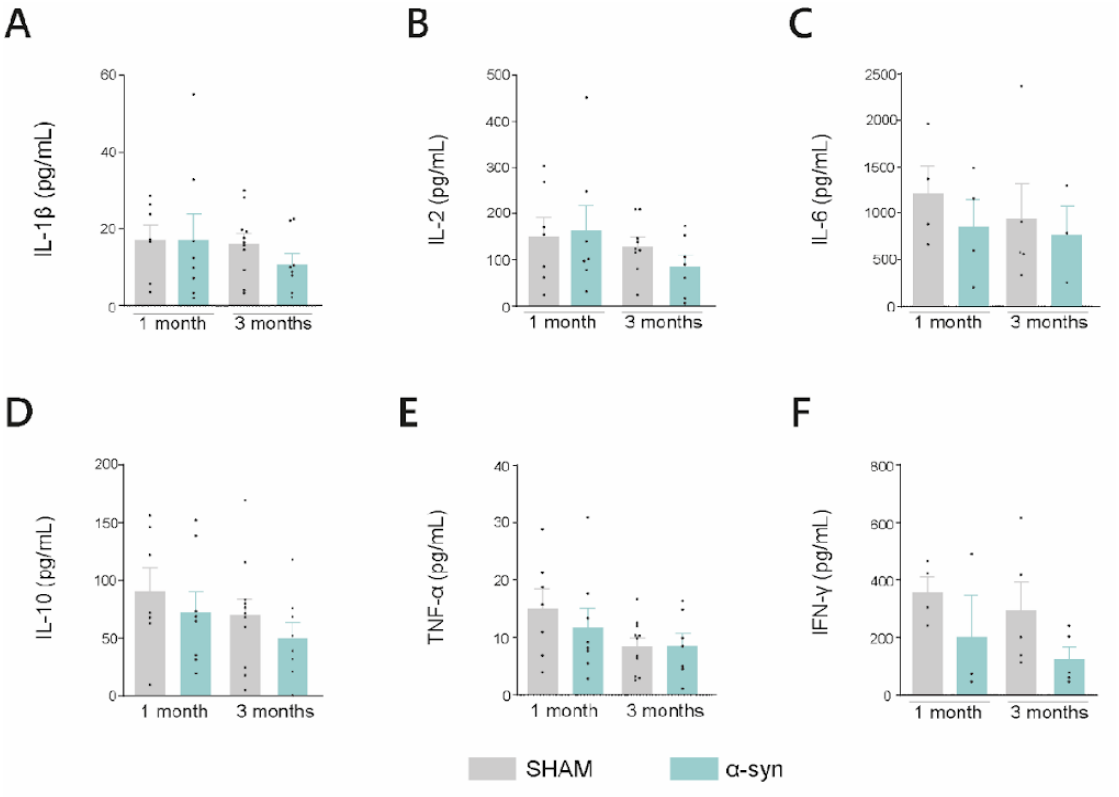
Statistical analysis of data

Experimental data were analysed using GraphPad v9.2.0 software (GraphPad Software Inc, U.S.A.). The level of statistical significance was set at $p<0.05$. All data were tested for outlier identification before statistical analysis and normality was checked using Shapiro-Wilk test with α of 0.05. Results are graphically presented as the group mean ± standard error of the mean (S.E.M.). Behavioural, immunohistochemical, mitochondrial activity and ELISA studies were analysed using 2-way ANOVA followed by post-hoc comparisons using Tukey´s test.

Supplementary Figures and Tables



Supplementary Fig. 1 Widespread of human α-syn along the brain. Representative images of analysed α-syn expression 3 months after the injection. (**A**) Bregma +1.92mm. (**B**) Bregma -2.16mm. (**C**) Bregma -3.60mm. (**D**) Bregma -4.68mm. (**E**) Bregma -7.92mm. (**F**) Bregma -10.08mm. **Acb:** accumbens nucleus; **BLA:** basolateral amygdala; **Cb:** cerebellum; **CeL:** central amygdala; **Cg2:** cingulate cortex, area 2; **CX:** cerebral cortex; **DR:** dorsal raphe nucleus; **GP:** globus pallidus; **Inf. Col:** inferior colliculus; **LC:** locus coeruleus; **LSI:** lateral septal nucleus; **MD:** mediodorsal thalamic nucleus; **Pn:** pontine nucleus; **PnC:** pontine nucleus, caudal part; **Po:** posterior thalamic nuclear group; **SNpc:** susbstantia nigra pars compacta; **SNpr:** substantia nigra pars reticulate; **STN:** subthalamic nucleus; **Sup. Col:** superior colliculus; **SuVe:** superior vestibular nucleus; **VPM:** ventral posteriomedial thalamic nucleus; **ZI:** zona incerta.



Supplementary Fig. 2 Inflammatory markers in serum. (A) IL-1β levels in serum. (B) IL-2 levels in serum. (C) IL-6 levels in serum. (D) IL-10 levels in serum. (E) TNF-α levels in serum. (F) IFN-γ levels in serum. No significant changes were observed in any of the studied markers. Data are presented as mean ± SEM of n= 3-11 animals per group.

Supplementary Table 1 Antibodies used in immunohistochemical assays.

Antibody	Activity	Dilution	Reference	Purchased from
Rabbit anti-TH	Primary antibody	1:2000	AB152	Abcam
Mouse anti-TH	Primary antibody	1:2000	AB5280	Abcam
Mouse anti-α-syn	Primary antibody	1:10000	AB27766	Abcam
Rabbit anti-α-synP	Primary antibody	1:2000	AB51253	Abcam
Biotinylated goat anti-rabbit IgG	Secondary antibody	1:200	BA100	VECTOR laboratories
Biotinylated goat anti-mouse IgG	Secondary antibody	1:200	BA2020	VECTOR laboratories
Goat anti-rabbit Alexa fluor 488	Secondary antibody	1:400	A11008	Invitrogen
Donkey anti-mouse Alexa fluor 555	Secondary antibody	1:400	A31570	Invitrogen

Supplementary Table 2 Assay solutions and incubation times for mitochondrial activity assays.

		Brain (CMMAs)	Platelets and PBMCs (CMAs)
Complex I	**Basal**	0.05mg/mL NBT, 1mM NADH, 0.005% cytC, 5mM PB (pH 7.4)	0.1mg/mL NBT, 1mM NADH, 2mM NAD^+, 0.005% cytC, 5mM PB (pH 7.4)
	Maximum	0.05mg/mL NBT, 1mM NADH, 0.005% cytC, 5mM PB (pH 7.4), 10mM sodium azide, 50µM duQ	0.1mg/mL NBT, 1mM NADH, 2mM NAD^+, 0.005% cytC, 5mM PB (pH 7.4), 10mM sodium azide, 50µM duQ
	Incubation time	7h	4h for platelets and 2h for PBMCs
Complex II	**Basal**	0.1mg/mL NBT, 10mM succinate, 0.01% cytC, 0.1M PB (pH 7.4)	0.1mg/mL NBT, 10mM succinate, 0.01% cytC, 0.1M PB (pH 7.4)
	Maximum	0.1mg/mL NBT, 10mM succinate, 0.01% cytC, 0.1M PB (pH 7.4), 5µM antimycin A, 50µM duQ	0.1mg/mL NBT, 10mM succinate, 0.01% cytC, 0.1M PB (pH 7.4), 5µM antimycin A, 50µM duQ
	Incubation time	1.5h	3h for platelets and 5h for PBMCs
GAPDH	**Basal**	0.1mg/mL NBT, 10mM G3P, 0.01% cytC, 0.1M PB (pH 7.4)	0.1mg/mL NBT, 10mM G3P, 0.01% cytC, 0.1M PB (pH 7.4)
	Maximum	0.1mg/mL NBT, 10mM G3P, 0.01% cytC, 0.1M PB (pH 7.4), 5µM antimycin A, 50µM duQ	0.1mg/mL NBT, 10mM G3P, 0.01% cytC, 0.1M PB (pH 7.4), 5µM antimycin A, 50µM duQ
	Incubation time	6h	2h for platelets and 3h for PBMCs
Complex IV	**Basal**	1.35mM DAB, 0.1M PB (pH 7.4)	1.35mM DAB, 0.1M PB (pH 7.4)
	Maximum	1.35mM DAB, 0.1M PB (pH 7.4), 0.01% cytC	1.35mM DAB, 0.1M PB (pH 7.4), 0.02% cytC
	Incubation time	2.5h	4h for platelets and 3h for PBMCs

Supplementary Table 3 Semiquantitative analysis of human α-syn expression. Occurrence of human α-syn positive axons was assessed as follows: - no positive axons, + few positive axons, ++ more positive axons, +++ positive axons densely covering the region, ++++ positive cell bodies.

a-syn expression				
			1 month	3 months
Bregma 4.20 mm	*Prefrontal cortex*	*Prefrontal cortex*	-	-
		Cingulate cortex 1	+	-
Bregma 2.28 mm	*Striatum*		+++	+++
	Accumbens nucleus	*Core*	+++	+++
		Shell	++	++
	Lateral septal nucleus		-	++
	Cortex	*Cortex*	-	-
		Cingulate cortex 2	+	++
Bregma 1.44 mm	*Striatum*		+++	+++
	Accumbens nucleus	*Core*	+++	+++
	Lateral septal nucleus		-	++
	Cortex	*Cortex*	-	+
		Cingulate cortex 2	-	++
Bregma 1.08 mm	*Striatum*		+++	+++
	Accumbens nucleus	*Core*	++	++
	Lateral septal nucleus		+	+
Bregma -0.48 mm	*Striatum*		+++	+++
	Lateral septal nucleus		-	++
	Globus pallidus		++	++
	Intermediate gray layer, superior colicullus	*Superficial gray layer*	+	++
		Lateral preoptic area	++	++
Bregma -1.44 mm	*Striatum*		+++	+++
	Globus pallidus		++	++
	Thalamus	*Reticular formation*	+++	+++
Bregma -2.04 mm	*Striatum*		+++	+++
	Globus pallidus		++	++
	Thalamus	*Reticular formation*	++	+++
		Ventral	+	++
	Zona incerta		+++	+++
	Amygdala	*Basolateral amygdala*	-	-
Bregma -2.52 mm	*Striatum*		+++	+++
	Globus pallidus		+	+
	Thalamus	*Internal capsule*	-	-
		Reticular formation	+	+
		Posterior	+	+
		Submedius	++	+++
	Amygdala	*Basolateral amygdala*	-	-
		Central amygdala	-	+
	Hippocampus		-	-

Supplementary Table 3 Semiquantitative analysis of human α-syn expression. Occurrence of human α-syn positive axons was assessed as follows: - no positive axons, + few positive axons, ++ more positive axons, +++ positive axons densely covering the region, ++++ positive cell bodies.

a-syn expression				
			1 month	3 months
Bregma -3.60 mm	*Striatum*		+++	+++
	Thalamus	*Posterior*	+	+
		Mediodorsal	++	++
	Zona incerta	*Dorsal*	+++	+++
		Ventral	++	++
	Subthalamic nucleus		+++	+++
	Amygdala	*Central amygdala*	-	+
	Hippocampus		-	-
Bregma -4.36 mm	*Thalamus*	*Posterior*	-	-
	ZI	*Dorsal*	+++	+++
		Ventral	++	++
	Substantia nigra	*Reticulata*	+++	+++
	Hippocampus		-	-
Bregma -4.68 mm	*Thalamus*	*Lateral posterior*	-	-
	Anterior protectal nucleus		++	++
	Zona incerta		++	++
	Substantia nigra	*Pars compacta*	++++	++++
		Reticulata	++	++
	Ventral tegmental area		++	+++
Bregma -5.64 mm	*Superior Colliculus*	*Superficial gray layer*	+	++
		Optic nerve layer	+	+
	Substantia nigra	*Pars compacta*	++++	++++
		Reticulata	+++	+++
Bregma -6.24 mm	*Superior Colliculus*	*Superficial gray layer*	++	++
		Optical nerve layer	+	+
		Intermediate layer	++	++
	Substantia nigra	*Pars Compacta*	++++	++++
		Reticulata	+++	+++
Bregma -7.92 mm	*Inferior colliculus*		-	-
	Dorsal raphe nucleus		++	+++
	Pontine nuclei		++	++
Bregma -10.08mm	*Cerebellum*		-	-
	Locus coeruleus		++	++
	Superior Vestibular nucleus		++	+++
	Pontine nuclei	*Caudal*	+	+

Supplementary Table 4 Obtained results of the predictive models.

Predictive model			
	AUC	**Accuracy**	**Precision**
Neural network	0.828	0.750	0.752
Logistic regression	0.816	0.778	0.778

Supplementary Table 5 Summary of statistical analysis of the behavioural evaluation. Parametric test, two-way ANOVA. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Two way ANOVA F (DFn, DFd)				
		Interaction	**Age**	**Group (SHAM vs. α-syn)**
Open Field	Activity	F (1, 60) = 0.2849	F (1, 60) = 20.43****	F (1, 60) = 3.553
	Distance	F (1, 60) = 0.4650	F (1, 60) = 13.43***	F (1, 60) = 2.936
	Number of rearings	F (1, 60) = 0.1440	F (1, 60) = 26.05****	F (1, 60) = 3.791
	Permanence time in the centre of the arena	F (1, 60) = 0.0048	F (1, 60) = 1.986	F (1, 60) = 0.2259
Rotarod	Time on the rotarod	F (1, 59) = 1.564	F (1, 59) = 11.27**	F (1, 59) = 1.073
Catalepsy (Bar Test)	Time	F (1, 59) = 1.406	F (1, 59) = 0.1098	F (1, 59) = 0.0492
Elevated Plus Maze	Entries in the open arm	F (1, 39) = 0.3102	F (1, 39) = 0.9323	F (1, 39) = 0.7976
	Time in the open arm	F (1, 39) = 1.348	F (1, 39) = 1.751	F (1, 39) = 0.0505
Marble Burying Test	Buried marbles	F (1, 40) = 4.776*	F (1, 40) = 3.550	F (1, 40) = 0.0006

Supplementary Table 6 Summary of statistical analysis of the immunohistochemical verification. Parametric test, two-way ANOVA. *p<0.05, **p<0.01, ****p<0.0001.

Two way ANOVA F (DFn, DFd)				
		Interaction	**Age**	**Group (SHAM vs. α-syn)**
α-syn	Striatum (Optical density)	F (1, 32) = 3.511	F (1, 32) = 1.084	F (1, 32) = 853.5****
TH	Striatum (Optical density)			
	Rostral	F (1, 31) = 3.341	F (1, 31) = 0.6804	F (1, 31) = 0.0874
	Caudal	F (1, 31) = 3.079	F (1, 31) = 0.0064	F (1, 31) = 7.327*
	Total	F (1, 31) = 4.087	F (1, 31) = 4.934*	F (1, 31) = 8.332**
	Substantia nigra (Stereology)	F (1,24) = 5.585*	F (1,24) = 0.5002	F (1, 24) = 2.538

Supplementary Table 7 Summary of statistical analysis of the inflammation in serum assay. Parametric test, two-way ANOVA.

Two way ANOVA F (DFn, DFd)				
		Interaction	**Age**	**Group (SHAM vs. α-syn)**
Inflammatory markers	**IL-1β**	F(1, 30) = 0.4324	F (1, 30) = 0.9200	F (1, 30) = 0.4167
	IL-2	F (1, 26) = 0.5996	F (1, 26) = 2.002	F (1, 26) = 0.2155
	IL-6	F (1, 12) = 0.0788	F (1, 12) = 0.2781	F (1, 12) = 0.6353
	IL-10	F (1, 30) = 0.0013	F (1, 30) = 1.798	F (1, 30) = 1.359
	TNF-α	F (1, 29) = 0.4657	F (1, 29) = 4.088	F (1, 29) = 0.4223
	IFN-γ	F (1, 13) = 0.0087	F (1, 13) = 0.6840	F (1, 13) = 3.776

Supplementary Table 8 Summary of statistical analysis of the mitochondrial activity assays. Parametric test, two-way ANOVA. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Two way ANOVA F (DFn, DFd)				
		Interaction	**Age**	**Group (SHAM vs. α-syn)**
Complex I	*Substantia nigra*	F (1, 35) = 1.719	F (1, 35) = 1.445	F (1, 35) = 6.040*
	Striatum	F (1, 39) = 0.0011	F (1, 39) = 1.278	F (1, 39) = 0.2956
	Cortex	F (1, 40) = 4.198*	F (1, 40) = 0.7666	F (1, 40) = 3.282
	Platelets	F (1, 40) = 0.7011	F (1, 40) = 23.48****	F (1, 40) = 6.163*
	PBMCs	F (1,37) = 0.2700	F (1, 37) = 2.359	F (1, 37) = 2.740
Complex II	*Substantia nigra*	F (1, 38) = 0.0235	F (1, 38) = 0.1724	F (1, 38) = 0.5550
	Striatum	F (1, 39) = 1.386	F (1, 39) = 9.100**	F (1, 39) = 0.1875
	Cortex	F (1, 40) = 2.369	F (1, 40) = 0.0442	F (1, 40) = 0.8401
	Platelets	F (1, 39) = 0.1513	F (1, 39) = 15.66***	F (1, 39) = 1.301
	PBMCs	F (1, 32) = 1.894	F (1, 32) = 1.140	F (1, 32) = 7.128*
Glyceraldehyde 3-phosphate dehydrogenase	*Substantia nigra*	F (1, 39) = 0.1052	F (1, 39) = 0.2594	F (1, 39) = 0.7734
	Striatum	F (1, 40) = 0.0289	F (1, 40) = 17.42***	F (1, 40) = 5.930*
	Cortex	F (1, 40) = 6.080*	F (1, 40) = 1.071	F (1, 40) = 0.4823
	Platelets	F (1, 40) = 0.0119	F (1, 40) = 4.236*	F (1, 40) = 0.9576
	PBMCs	F (1, 36) = 0.8388	F (1, 36) = 0.0489	F (1, 36) = 0.0319
Complex IV	*Substantia nigra*	F (1, 39) = 0.4640	F (1, 39) = 0.2231	F (1, 39) = 0.00008
	Striatum	F (1, 40) = 4.093*	F (1, 40) = 4.202*	F (1, 40) = 3.091
	Cortex	F (1, 40) = 2.588	F (1, 40) = 0.0024	F (1, 40) = 0.9602
	Platelets	F (1, 39) = 0.0740	F (1, 39) = 2.083	F (1, 39) = 10.23**
	PBMCs	F (1, 37) = 2.226	F (1, 37) = 4.880*	F (1, 37) = 8.076**
Complex IV kinetic	Platelets (Slope)	F (1, 31) = 9.401*	F (1, 31) = 15.31**	F (1, 31) = 7.197*