ADDITIONAL FILE 4

Neurodegeneration and contralateral α-synuclein induction after intracerebral α-synuclein injections in the anterior olfactory nucleus of a Parkinson's disease A53T mouse model

Alicia Flores-Cuadrado[1], Daniel Saiz-Sanchez[1], Alicia Mohedano-Moriano[2], Alino Martinez-Marcos[1], Isabel Ubeda-Bañon[1]*

[1]Neuroplasticity and Neurodegeneration Laboratory, CRIB, Ciudad Real Medical School, University of Castilla-La Mancha, Ciudad Real, Spain.
[2]School of Occupational Therapy, Speech Therapy and Nursing, University of Castilla-La Mancha, Talavera de la Reina, Spain.

Alicia.flores@uclm.es
Daniel.saiz@uclm.es
Alicia.mohedano@uclm.es
Alino.martinez@uclm.es

Address for correspondence:
Isabel Ubeda-Bañon
University of Castilla-La Mancha
Ciudad Real Medical School
Camino de Moledores s/n
13071 Ciudad Real (Spain)
Phone: 926295300 6835
E-mail Isabel.ubeda@uclm.es

Stereological α-synuclein, NeuN, Iba-1, GFAP quantification

Table S8. Statistical data of stereological α-synuclein quantification.

Area	Group (TG mice)	Comparisons (cells/mm^3)	t-test	P value
OB	Saline-injection	Left hemisphere × Right hemisphere	$t_6 = 6.948$	P = 0.0004***
	α-syn-injection	Left hemisphere × Right hemisphere	$t_4 = 1.605$	P = 0.1838
	α-syn-injection x Saline-injection	Right hemisphere × Right hemisphere	$t_5 = 2.080$	P = 0.0921
	α-syn-injection x Saline-injection	Left hemisphere × Left hemisphere	$t_5 = 3.805$	P = 0.0126*
AON	Saline-injection	Left hemisphere × Right hemisphere	$t_6 = 3.197$	P = 0.0187*
	α-syn-injection	Left hemisphere × Right hemisphere	$t_4 = 1.315$	P = 0.2588
	α-syn-injection x Saline-injection	Right hemisphere × Right hemisphere	$t_5 = 2.310$	P = 0.0689
	α-syn-injection x Saline-injection	Left hemisphere × Left hemisphere	$t_5 = 2.104$	P = 0.0893
Pir	Saline-injection	Left hemisphere × Right hemisphere	$t_6 = 0.334$	P = 0.7493
	α-syn-injection	Left hemisphere × Right hemisphere	$t_4 = 0.115$	P = 0.9136
	α-syn-injection x Saline-injection	Right hemisphere × Right hemisphere	$t_5 = 2.225$	P = 0.0767
	α-syn-injection x Saline-injection	Left hemisphere × Left hemisphere	$t_5 = 2.514$	P = 0.0536

Table S9. Statistical data of NeuN quantification (Mann-Whitney test).

Area	Source of variation	P value
OB	WT-saline-RH x TG-saline-RH	P = 0.0286*
	WT-saline-LH x TG-saline-LH	P = 0.0286*
	WT-α-RH x TG-α-RH	P = 0.2286
	WT-α-LH x TG-α-LH	P = 0.1143
	TG-saline-RH x TG-α-RH	P = 0.0571
	TG-saline-LH x TG-α-LH	P = 0.0571
	WT-saline-RH x WT-α-RH	P = 0.4857
	WT-saline-LH x WT-α-LH	P = 0.1143
AON	WT-saline-RH x TG-saline-RH	P = 0.0286*
	WT-saline-LH x TG-saline-LH	P = 0.0286*
	WT-α-RH x TG-α-RH	P = 0.4000
	WT-α-LH x TG-α-LH	P = 0.0571
	TG-saline-RH x TG-α-RH	P = 0.0571
	TG-saline-LH x TG-α-LH	P = 0.0571
	WT-saline-RH x WT-α-RH	P = 0.4857
	WT-saline-LH x WT-α-LH	P = 0.8286
Pir	WT-saline-RH x TG-saline-RH	P = 0.0286*
	WT-saline-LH x TG-saline-LH	P = 0.0286*
	WT-α-RH x TG-α-RH	P > 0.9999
	WT-α-LH x TG-α-LH	P = 0.2286
	TG-saline-RH x TG-α-RH	P = 0.0571
	TG-saline-LH x TG-α-LH	P = 0.0571
	WT-saline-RH x WT-α-RH	P = 0.0286*
	WT-saline-LH x WT-α-LH	P > 0.9999

Table S10. Statistical data of stereological Iba-1 quantification. Comparison of genotype in the right hemisphere.

Area	Source of variation	F (DFn, DFd)	P value
GL	Interaction	F (1, 11) = 0.04697	P = 0.8324
	Treatment	F (1, 11) = 1.256	P = 0.2863
	Genotype	F (1, 11) = 0.3596	P = 0.5609
EPL	Interaction	F (1, 11) = 0.06955	P = 0.7969
	Treatment	F (1, 11) = 1.053	P = 0.3268
	Genotype	F (1, 11) = 1.448	P = 0.2541
MiL	Interaction	F (1, 11) = 4.943	P = 0.0481* (t)
	Treatment	F (1, 11) = 0.8695	P = 0.3711
	Genotype	F (1, 11) = 4.519	P = 0.0570
IPL	Interaction	F (1, 11) = 0.3797	P = 0.5503
	Treatment	F (1, 11) = 1.604	P = 0.2315
	Genotype	F (1, 11) = 0.02487	P = 0.8775
GrL	Interaction	F (1, 11) = 1.013	P = 0.3358
	Treatment	F (1, 11) = 3.076	P = 0.1072
	Genotype	F (1, 11) = 0.1085	P = 0.7480
AON	Interaction	F (1, 11) = 0.03820	P = 0.8486
	Treatment	F (1, 11) = 0.01318	P = 0.9107
	Genotype	F (1, 11) = 1.427	P = 0.2573
Pir	Interaction	F (1, 11) = 0.3274	P = 0.5787
	Treatment	F (1, 11) = 1.016	P = 0.3351
	Genotype	F (1, 11) = 0.03855	P = 0.8479

(t) TG-α-right x TG-S-right: $t_5 = 3.410$; P = 0.0190.

Table S11. Statistical data of stereological Iba-1 quantification. Comparison of genotype in the left hemisphere.

Area	Source of variation	F (DFn, DFd)	P value
GL	Interaction	F (1, 11) = 3.982	P = 0.0714
	Treatment	F (1, 11) = 0.1076	P = 0.7490
	Genotype	F (1, 11) = 0.05624	P = 0.8169
EPL	Interaction	F (1, 11) = 0.1320	P = 0.7233
	Treatment	F (1, 11) = 2.039	P = 0.1811
	Genotype	F (1, 11) = 1.927	P = 0.1925
MiL	Interaction	F (1, 11) = 3.576	P = 0.0852 (t1)
	Treatment	F (1, 11) = 1.189	P = 0.2989
	Genotype	F (1, 11) = 0.7315	P = 0.4106
IPL	Interaction	F (1, 11) = 4.346e-005	P = 0.9949
	Treatment	F (1, 11) = 2.225	P = 0.1639
	Genotype	F (1, 11) = 0.2819	P = 0.6060
GrL	Interaction	F (1, 11) = 2.116	P = 0.1737
	Treatment	F (1, 11) = 0.004102	P = 0.9501
	Genotype	F (1, 11) = 0.3776	P = 0.5514
AON	Interaction	F (1, 11) = 0.7252	P = 0.4126
	Treatment	F (1, 11) = 0.2762	P = 0.6096
	Genotype	F (1, 11) = 0.002602	P = 0.9602
Pir	Interaction	F (1, 11) = 0.4845	P = 0.5008
	Treatment	F (1, 11) = 0.6570	P = 0.4348 (t2)
	Genotype	F (1, 11) = 0.05178	P = 0.8242

(t1) TG-α-left × TG-S-left: $t_5 = 3.626$; P = 0.0151.

(t2) TG-α-left × TG-S-left: $t_5 = 2.620$; P = 0.0471.

Table S12. Statistical data of stereological Iba-1 quantification. Comparison of hemispheres in WT.

Area	Source of variation	F (DFn, DFd)	P value
GL	Interaction	F (3, 26) = 1.253	P = 0.3108
	Treatment × Time	F (3, 26) = 52.42	P < 0.0001****
	Hemisphere	F (1, 26) = 0.01173	P = 0.9146
EPL	Interaction	F (3, 26) = 1.470	P = 0.2456
	Treatment × Time	F (3, 26) = 82.29	P < 0.0001****
	Hemisphere	F (1, 26) = 0.2745	P = 0.6047
MiL	Interaction	F (3, 26) = 0.1273	P = 0.9431
	Treatment × Time	F (3, 26) = 39.19	P < 0.0001****
	Hemisphere	F (1, 26) = 0.1012	P = 0.7530
IPL	Interaction	F (3, 26) = 0.1904	P = 0.9020
	Treatment × Time	F (3, 26) = 36.83	P < 0.0001****
	Hemisphere	F (1, 26) = 0.05854	P = 0.8107
GrL	Interaction	F (3, 26) = 1.304	P = 0.2944
	Treatment × Time	F (3, 26) = 149.7	P < 0.0001****
	Hemisphere	F (1, 26) = 1.179	P = 0.2876
AON	Interaction	F (3, 26) = 1.304	P = 0.2944
	Treatment × Time	F (3, 26) = 149.7	P < 0.0001****
	Hemisphere	F (1, 26) = 1.179	P = 0.2876
Pir	Interaction	F (3, 26) = 0.06495	P = 0.9779
	Treatment × Time	F (3, 26) = 35.75	P < 0.0001****
	Hemisphere	F (1, 26) = 0.001407	P = 0.9704

Table S13. Statistical data of stereological Iba-1 quantification. Comparison of hemispheres in TG.

Area	Source of variation	F (DFn, DFd)	P value
GL	Interaction	F (1, 10) = 0.1615	P = 0.6962
	Treatment	F (1, 10) = 2.842	P = 0.1227
	Hemisphere	F (1, 10) = 0.3787	P = 0.5520
EPL	Interaction	F (1, 10) = 1.320	P = 0.2774
	Treatment	F (1, 10) = 0.006174	P = 0.9389
	Hemisphere	F (1, 10) = 0.9160	P = 0.3611
MiL	Interaction	F (1, 10) = 0.1243	P = 0.7318
	Treatment	F (1, 10) = 24.35	P = 0.0006***
	Hemisphere	F (1, 10) = 3.098	P = 0.1089
IPL	Interaction	F (1, 10) = 0.08663	P = 0.7745
	Treatment	F (1, 10) = 1.997	P = 0.1880
	Hemisphere	F (1, 10) = 0.06282	P = 0.8072
GrL	Interaction	F (1, 10) = 0.3451	P = 0.5699
	Treatment	F (1, 10) = 3.261	P = 0.1011
	Hemisphere	F (1, 10) = 0.001080	P = 0.9744
AON	Interaction	F (1, 10) = 0.1234	P = 0.7326
	Treatment	F (1, 10) = 0.002316	P = 0.9626
	Hemisphere	F (1, 10) = 0.2034	P = 0.6616
Pir	Interaction	F (1, 10) = 7.081	P = 0.0239* (t)
	Treatment	F (1, 10) = 0.007362	P = 0.9333
	Hemisphere	F (1, 10) = 0.07785	P = 0.7859

(t) TG-α-left × TG-S-left: $t_5 = 2.620$; P = 0.0471.

Table S14. Statistical data of GFAP quantification. Comparison of genotype in the right hemisphere.

Area	Source of variation	F (DFn, DFd)	P value
OB	Interaction	F (1, 11) = 0.2590	P = 0.6208
	Treatment	F (1, 11) = 0.6954	P = 0.4221
	Genotype	F (1, 11) = 32.13	P = 0.0001***
AON	Interaction	F (1, 11) = 1.121	P = 0.3123
	Treatment	F (1, 11) = 3.740	P = 0.0793
	Genotype	F (1, 11) = 11.73	P = 0.0057** (t)
Pir	Interaction	F (1, 11) = 0.3859	P = 0.5471
	Treatment	F (1, 11) = 0.3573	P = 0.5621
	Genotype	F (1, 11) = 3.034	P = 0.1094

(t) WT saline 2m-TG saline 2m: $t_6 = 3.134$; P = 0.0202.

Table S15. Statistical data of GFAP quantification. Comparison of genotype in the left hemisphere.

Area	Source of variation	F (DFn, DFd)	P value
OB	Interaction	F (1, 11) = 0.9921	P = 0.3406
	Treatment	F (1, 11) = 0.08698	P = 0.7735
	Genotype	F (1, 11) = 22.10	P = 0.0006***
AON	Interaction	F (1, 11) = 0.8663	P = 0.3720
	Treatment	F (1, 11) = 0.03401	P = 0.8570
	Genotype	F (1, 11) = 30.14	P = 0.0002***
Pir	Interaction	F (1, 11) = 1.283	P = 0.2814
	Treatment	F (1, 11) = 0.4568	P = 0.5131
	Genotype	F (1, 11) = 1.287	P = 0.2807

Table S16. Statistical data of GFAP quantification. Comparison of hemispheres in WT.

Area	Source of variation	F (DFn, DFd)	P value
OB	Interaction	F (3, 26) = 0.2841	P = 0.8364
	Treatment	F (3, 26) = 4.233	P = 0.0146*
	Hemisphere	F (1, 26) = 1.315	P = 0.2619
AON	Interaction	F (3, 26) = 0.05543	P = 0.9824
	Treatment	F (3, 26) = 2.106	P = 0.1239
	Hemisphere	F (1, 26) = 0.5590	P = 0.4614
Pir	Interaction	F (3, 26) = 1.279	P = 0.3024
	Treatment	F (3, 26) = 2.273	P = 0.1038
	Hemisphere	F (1, 26) = 0.6240	P = 0.4367

Table S17. Statistical data of GFAP quantification. Comparison of hemispheres in TG.

Area	Source of variation	F (DFn, DFd)	P value
OB	Interaction	F (1, 10) = 0.9984	P = 0.3413
	Treatment	F (1, 10) = 0.009101	P = 0.9259
	Hemisphere	F (1, 10) = 0.6813	P = 0.4284
AON	Interaction	F (1, 10) = 3.763	P = 0.0811
	Treatment	F (1, 10) = 2.085	P = 0.1793
	Hemisphere	F (1, 10) = 0.5549	P = 0.4735
Pir	Interaction	F (1, 10) = 0.03984	P = 0.8458
	Treatment	F (1, 10) = 1.002	P = 0.3405
	Hemisphere	F (1, 10) = 0.05028	P = 0.8271

1.1 Supplementary Figures

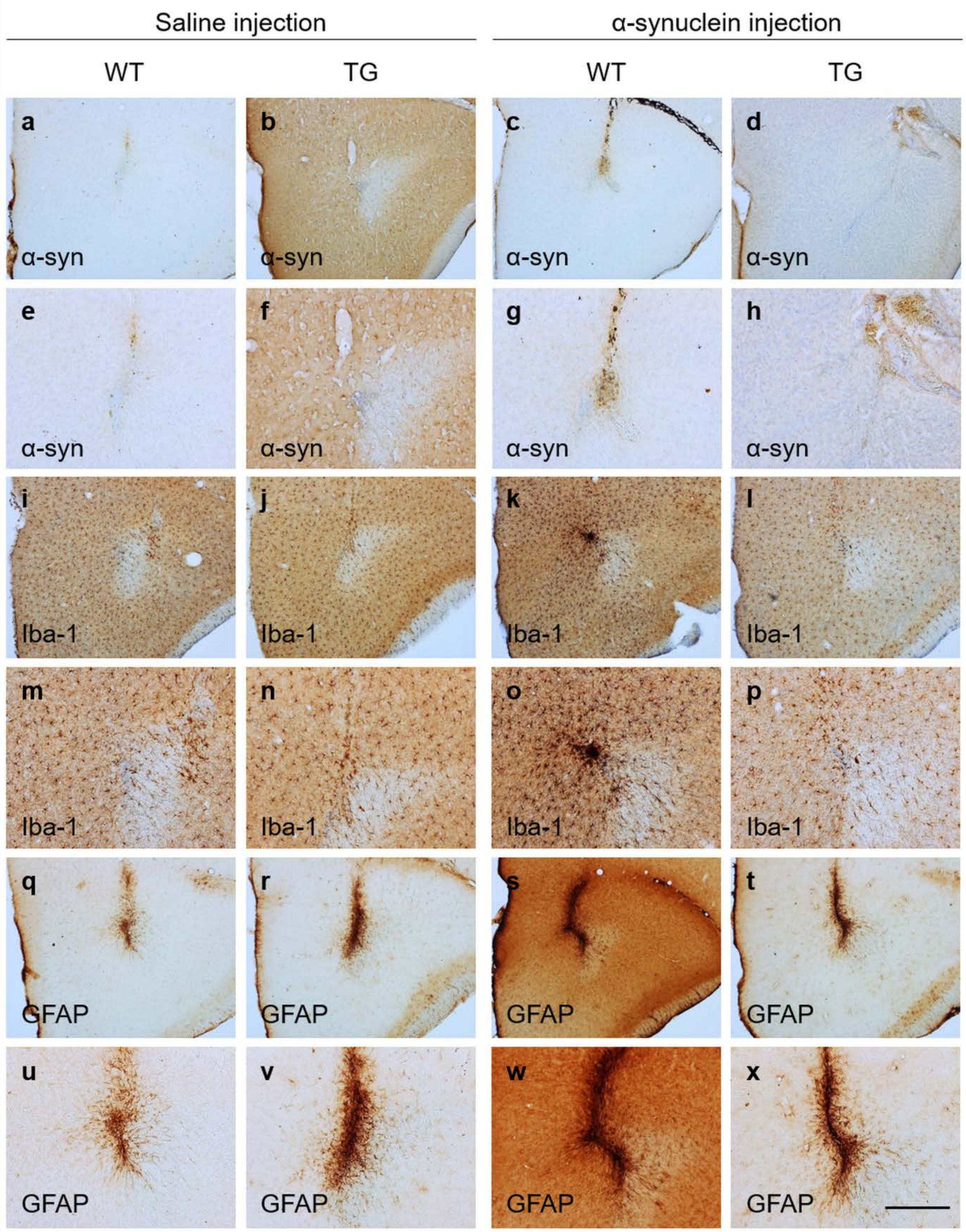

Fig. S6 AON injection site labeled with different markers. α-synuclein (**a**-**h**), Iba-1 (**i**-**p**) and GFAP (**q**-**x**). Scale bars: **a**-**d**, **i**-**l**, **q**-**t**, 500 μm; **e**-**h**, **m**-**p**, **u**-**x** 250 μm. For abbreviations, see list.

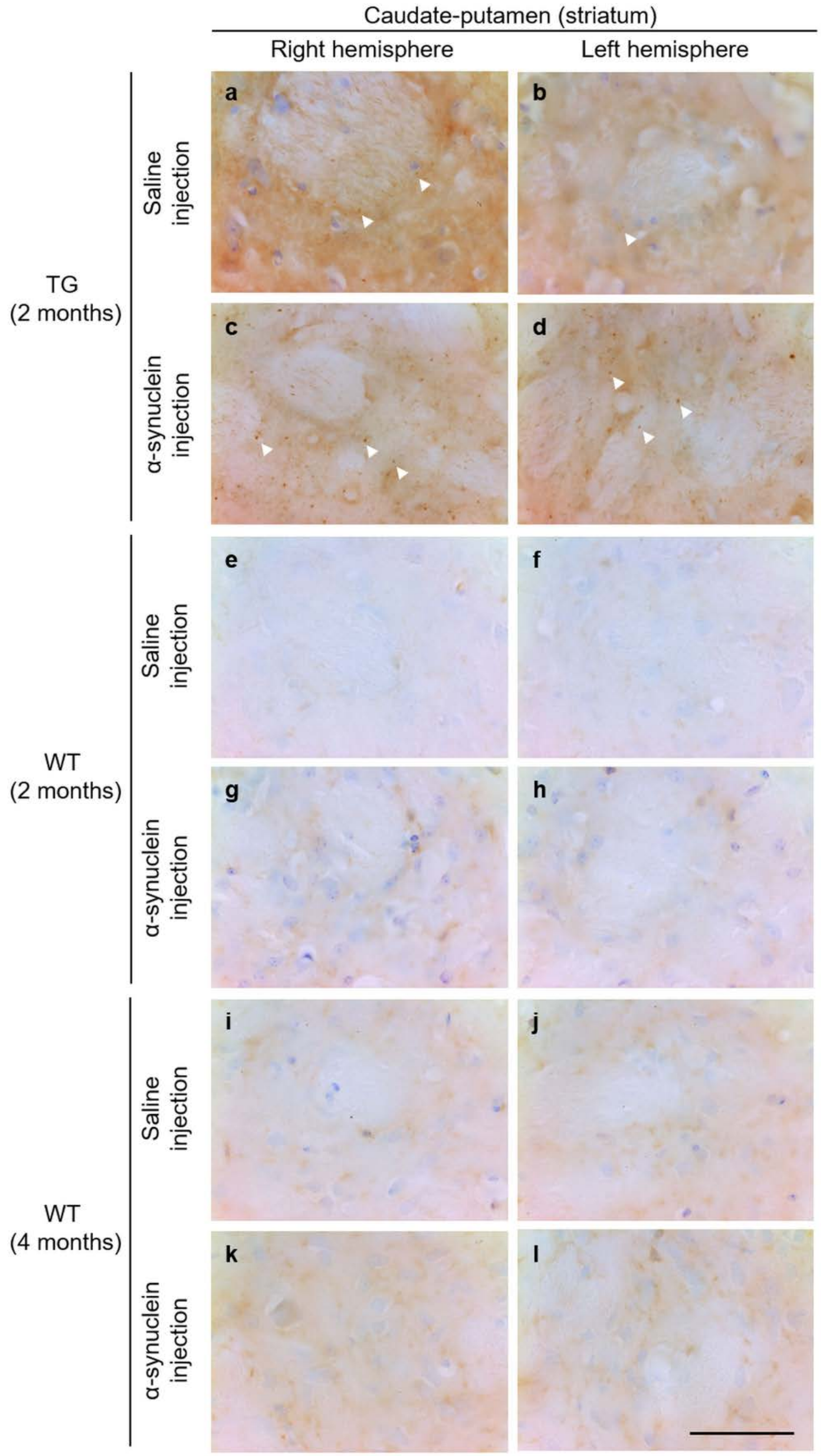

Fig. S7 α-synucleinopathy in the caudate-putamen (**a**-**l**). Arrows indicate α-synuclein aggregates around striosomes (organization of afferent and efferent fibers in the striatum) (**a**-**d**). These aggregates were not found in saline-injected and α-synuclein-injected WT animals (**e**-**l**). Scale bars: 50 μm.

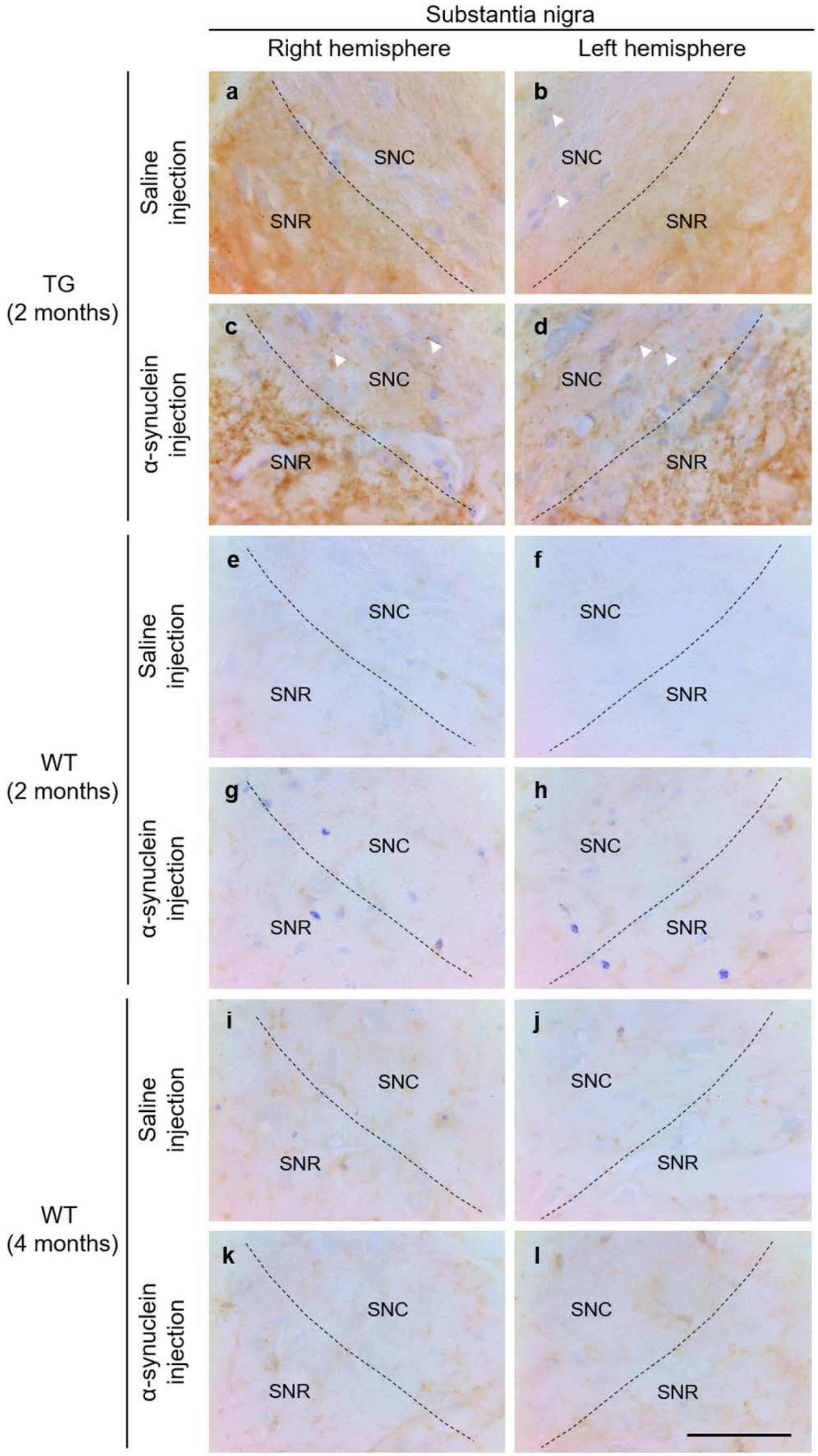

Fig. S8 α-synucleinopathy in the substantia nigra (**a-l**). Arrows indicate α-synuclein aggregates, mainly in substantia nigra compact part (SNC) (**a-d**). These aggregates were not observed in saline-injected and α-synuclein-injected WT animals (**e-l**). Scale bars: 50 µm.