Biondetti et al. Supplementary Material

Supplementary Table 1 Magnetic resonance imaging protocols

	T1-weighted anatomical MRI	**T1-weighted neuromelanin-sensitive MRI**	**T2*-weighted iron-sensitive MRI**
Protocol	3D MP2RAGE	2D TSE	3D FLASH
Slice orientation	Sagittal	Transverse, perpendicular to the longitudinal axis of the brainstem	Transverse
Phase encoding direction	Anterior-posterior	Anterior-posterior	Right-left
TE (ms)	2·98	13	4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 34 and 37
TR (ms)	5000	890	40
Echo train length	n/a	3	12
TI (ms)	700 and 2500	n/a	n/a
Flip angle (degrees)	4 and 5	90 and 180 (refocus)	20
Bandwidth (Hz/pixel)	240	160	1000
FoV: anterior-posterior x right-left x superior-inferior (mm3)	256 x 231 x 176	200 x 220 x 48	192 x 174 x 160
Voxel size (mm3)	1 x 1 x 1	0·43 x 0·43 x 3	1 x 1 x 2 reconstructed as 1 isotropic
Flow compensation	n/a	Slice-select direction	First-echo only, all encoding directions
Acquisition time (min:s)	08:12	06:55	09:18

Parameter settings for all the MRI sequences
FLASH = fast low angle shot; FoV = field of view; MP2RAGE = magnetization prepared two rapid acquisition gradient echoes; n/a = not applicable; TE = echo time; TI = inversion time; TR = repetition time; TSE = turbo spin echo

Supplementary results

Following MRI quality control, we excluded neuromelanin-sensitive images acquired in one HC, two iRBD patients and five Parkinson's disease patients at V1, and two PDs at V2, mostly because of incorrect field of view placement excluding part of the substantia nigra pars compacta (SNc) from the image. Iron-sensitive images were unavailable in ten HCs, five iRBD patients and twenty-two Parkinson's disease patients at V1, and one HC, seven iRBD patients and twenty-two Parkinson's disease patients at V2.

The unavailability of some iron-sensitive MRI data sets was caused by errors in raw data transmission from the MRI system to the image reconstruction software. Raw data reconstruction was required for quantitative susceptibility mapping (QSM), which exploits the MRI signal phase, because the Siemens default multi-channel combination procedure

calculates the MRI phase incorrectly. This is a well-known issue in the QSM community.[1] Several solutions have been developed to overcome this issue, such as the ad-hoc reconstruction method implemented in our imaging center.[1, 2] However, raw data retrieval is more susceptible to data communication issues from the MRI system. For future studies, we aim to try and retrieve these missing data.

For group difference analysis, all participants were included, as each contributed at least one measurement. For correlation analysis, only participants with corresponding complete pairs of measurements were included. The number of subjects retained for each analysis is listed in Supplementary Table 2.

Supplementary Table 2 Subjects retained for the analyses following MRI quality control

	HCs		**iRBDs**		**PDs**	
	V1	**V2**	**V1**	**V2**	**V1**	**V2**
Group difference / temporal fitting analyses:						
Neuromelanin	54	28	41	21	130	81
Iron	45	27	38	14	113	61
DaT	35	18	32	15	48	40
Correlation analyses:						
DaT-neuromelanin	n/a	n/a	30	n/a	46	n/a
DaT-iron	n/a	n/a	28	n/a	43	n/a
Neuromelanin-iron	n/a	n/a	35	n/a	110	n/a

For each subject group and visit, the table shows the number of subjects retained for each analysis. Group difference and temporal fitting analyses only required one measurement in each subject. Correlation analyses required pairs of measurements in each subject. Notably, correlation analyses were only performed on subjects at V1

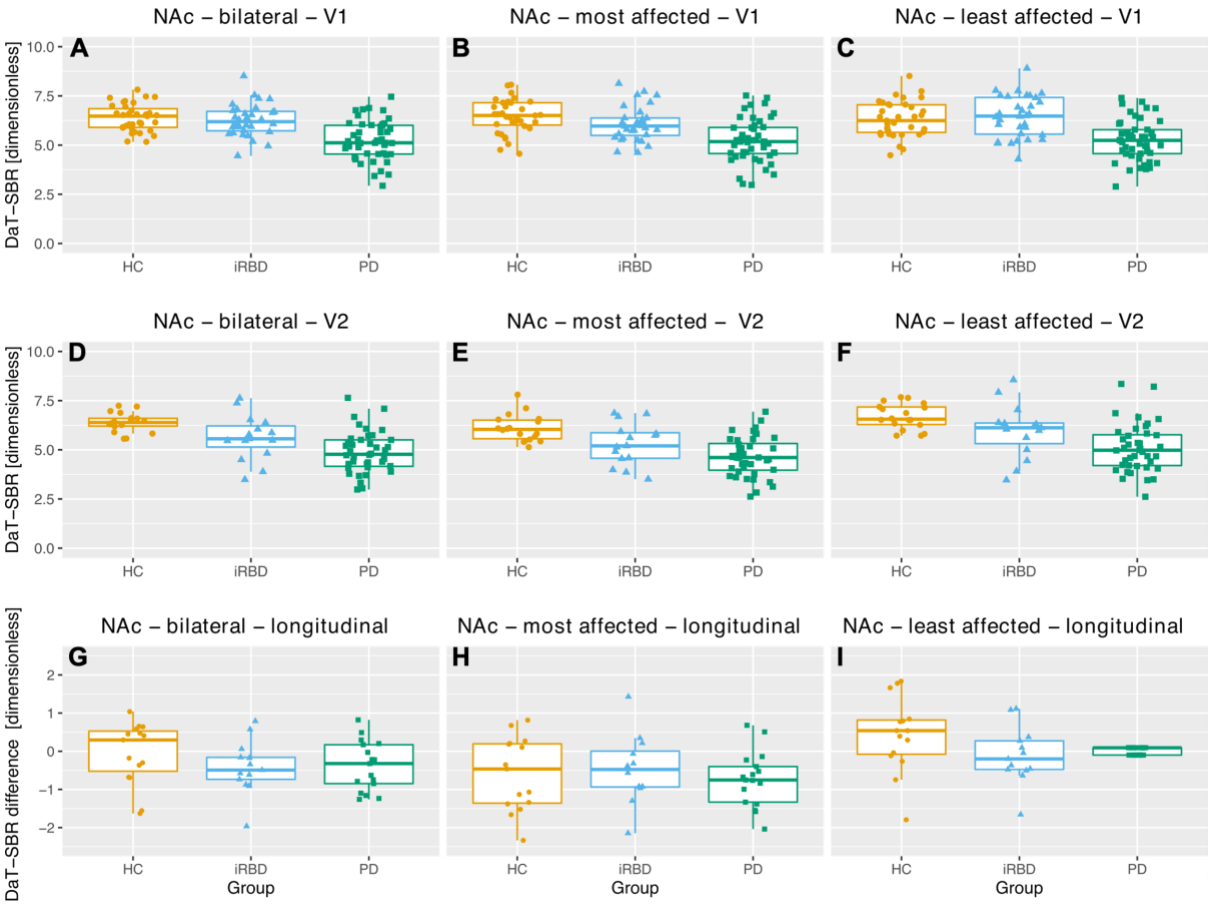
DaT = dopamine transporter; HC = healthy control subject; iRBD = idiopathic REM sleep behaviour disorder; n/a = not applicable; PD = Parkinson's disease; V1/V2 = visit one or two

Supplementary Table 3 Estimated loss in DaT SBR over disease duration

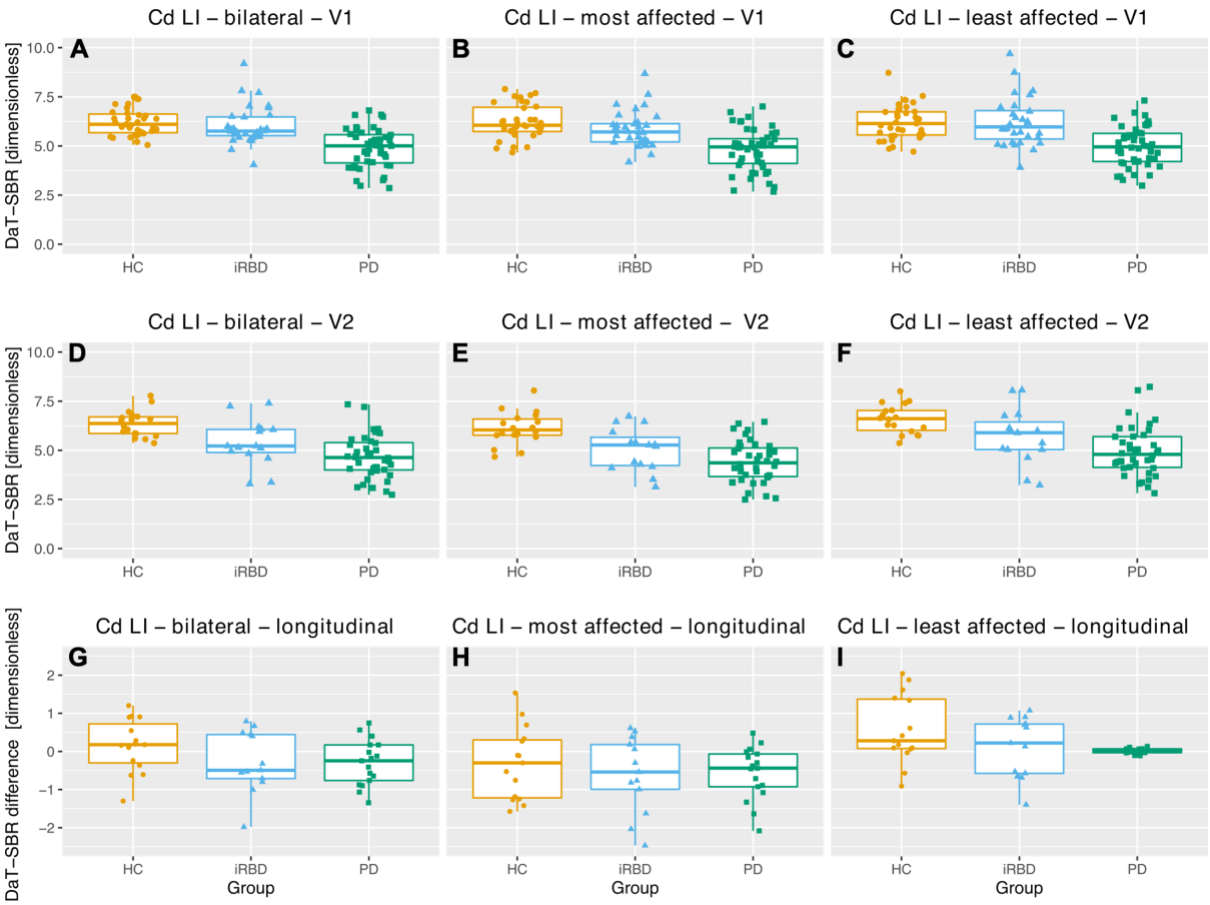
	Most affected hemisphere			**Least affected hemisphere**		
	Estimated DaT-SBR loss at PD onset [%]	Estimated beginning of the prodromal phase in PD [years]	Estimated iRBD time placement [years]	Estimated DaT-SBR loss at PD onset [%]	Estimated beginning of the prodromal phase in PD [years]	Estimated iRBD time placement [years]
NAc	8.0	-1.1	0.1	9.0	-1.8	-1.3
Limbic Cd	12.7	-1.9	-0.4	12.5	-3.2	-2.1
Associative Cd	23.6	-4.4	-2.6	13.9	-2.3	-1.4
Sensorimotor Cd	27.1	-5.6	-3.8	17.0	-3.0	-1.7
Limbic Pu	29.7	-6.1	-4.4	19.6	-3.4	-2.9
Associative Pu	48.0	-12.7	-10.3	34.8	-5.6	-4.6
Sensorimotor Pu	54.5	-20.7	-16.5	42.2	-7.8	-6.0

Cd = caudate nucleus; DaT = dopamine transporter; iRBD = idiopathic REM sleep behaviour disorder; NAc = nucleus accumbens; PD = Parkinson's disease; Pu = putamen; SBR = striatal binding ratio

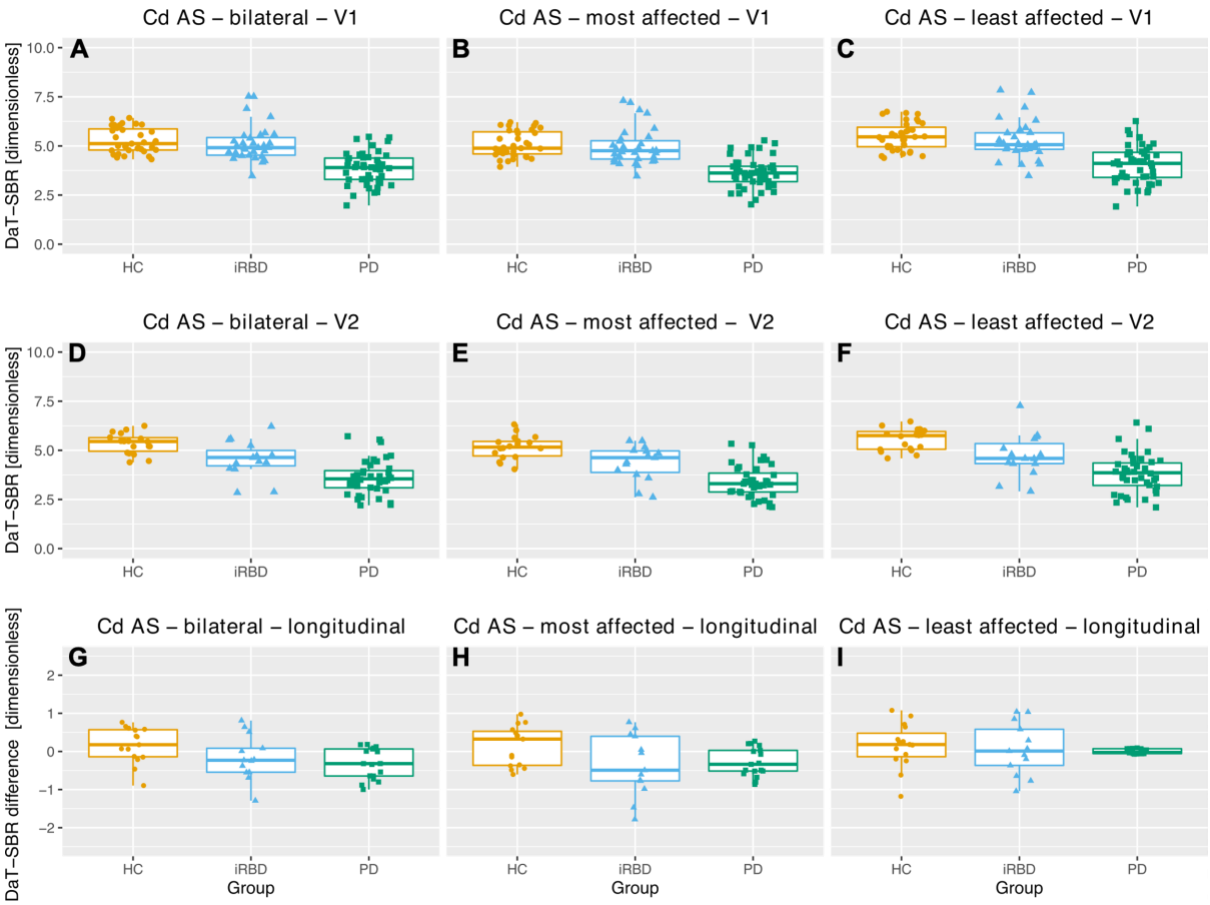
Supplementary figures



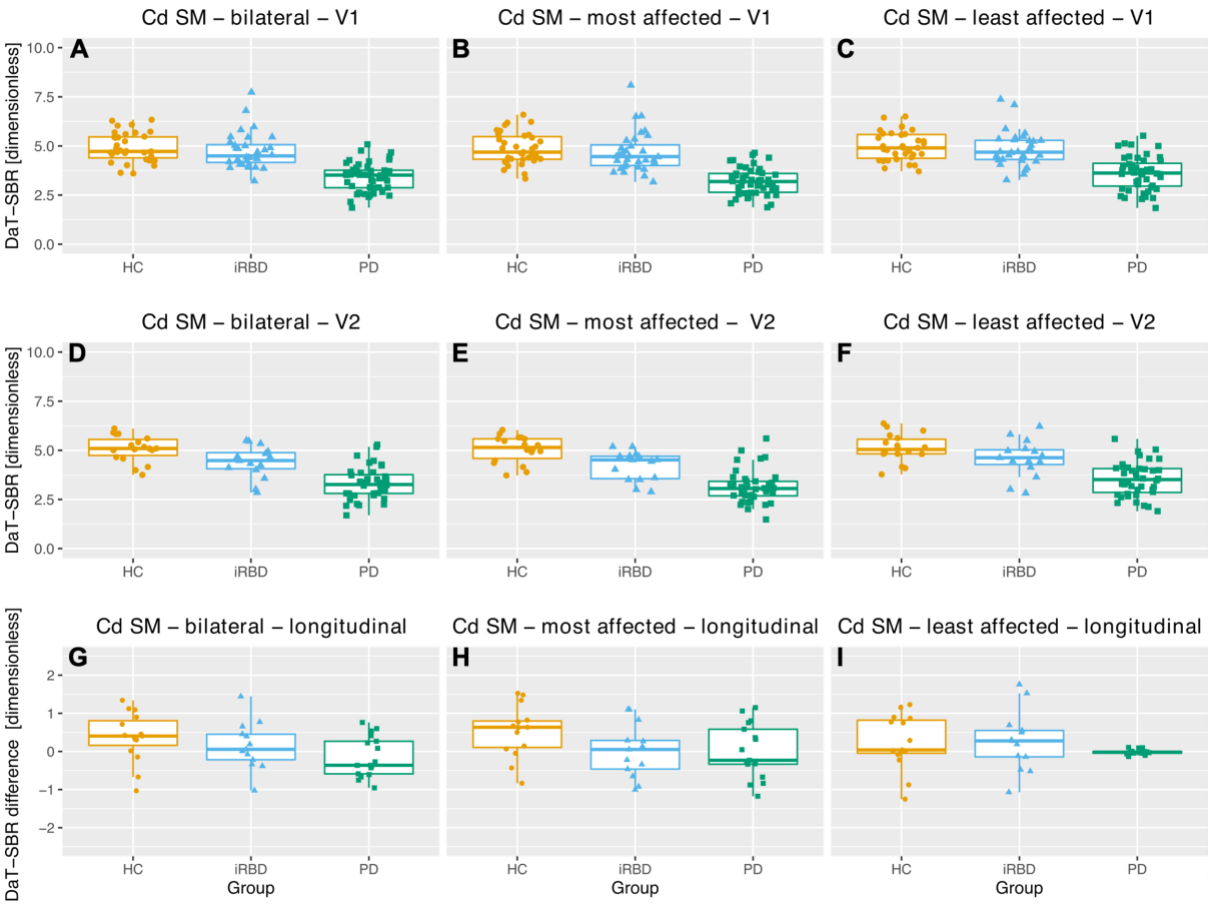
Supplementary Figure 1 Scatter boxplots of age and sex adjusted DaT-SBR measurements in the nucleus accumbens. **(A-C)** DaT-SBR at V1. **(D-F)** DaT-SBR at V2. **(G-I)** DaT-SBR difference in longitudinal examinations (V2 – V1). The left column shows bilateral values. The centre and right columns respectively show values in the clinically most and least affected brain hemispheres
DaT = dopamine transporter; HC = healthy control; iRBD = idiopathic REM sleep behaviour disorder; NAc = nucleus accumbens; PD = Parkinson's disease; SBR = striatal binding ratio; V1/V2 = visit 1/2



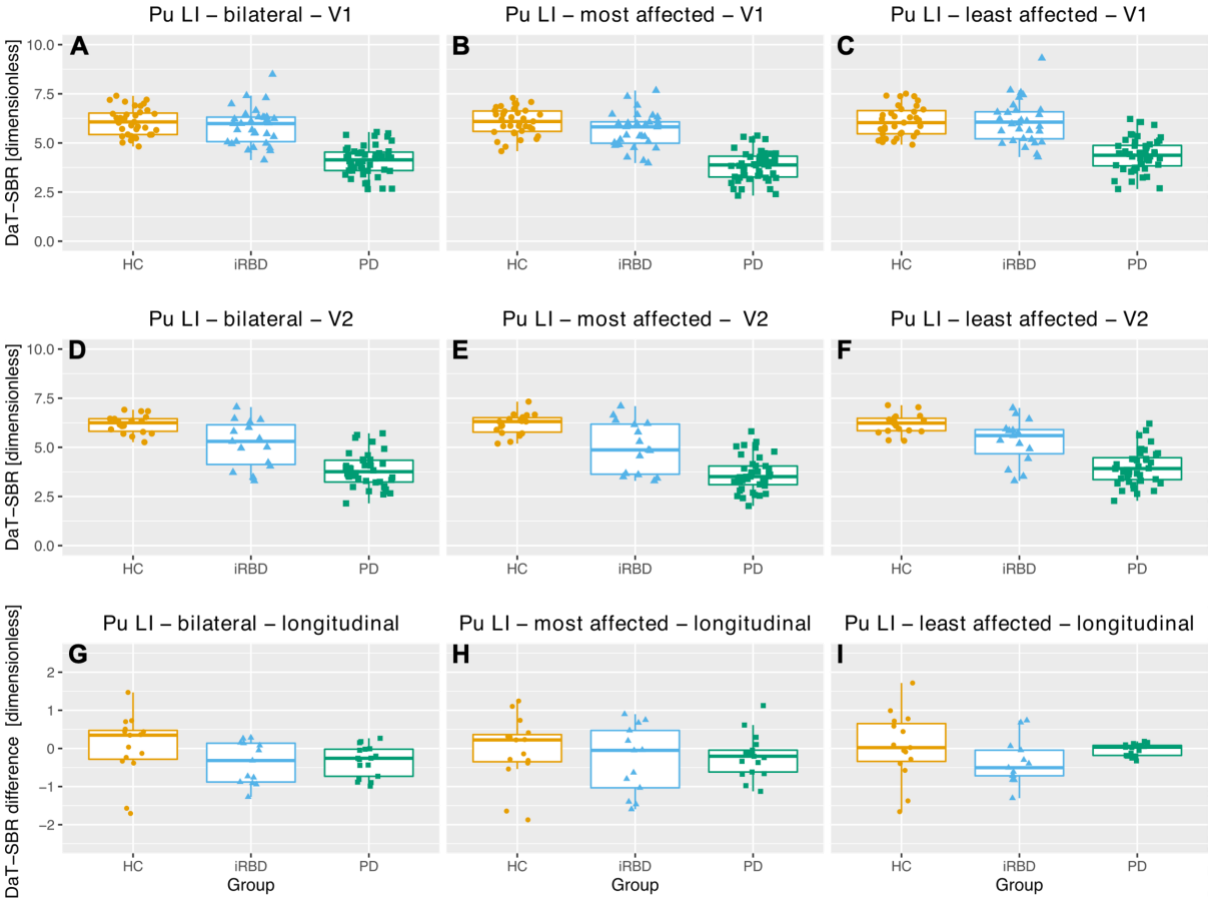
Supplementary Figure 2 Scatter boxplots of age and sex adjusted DaT-SBR measurements in the limbic caudate nucleus. **(A-C)** DaT-SBR at V1. **(D-F)** DaT-SBR at V2. **(G-I)** DaT-SBR difference in longitudinal examinations (V2 – V1). The left column shows bilateral values. The centre and right columns respectively show values in the clinically most and least affected brain hemispheres
Cd = caudate nucleus; DaT = dopamine transporter; HC = healthy control; iRBD = idiopathic REM sleep behaviour disorder; LI = limbic; PD = Parkinson's disease; SBR = striatal binding ratio; V1/V2 = visit 1/2



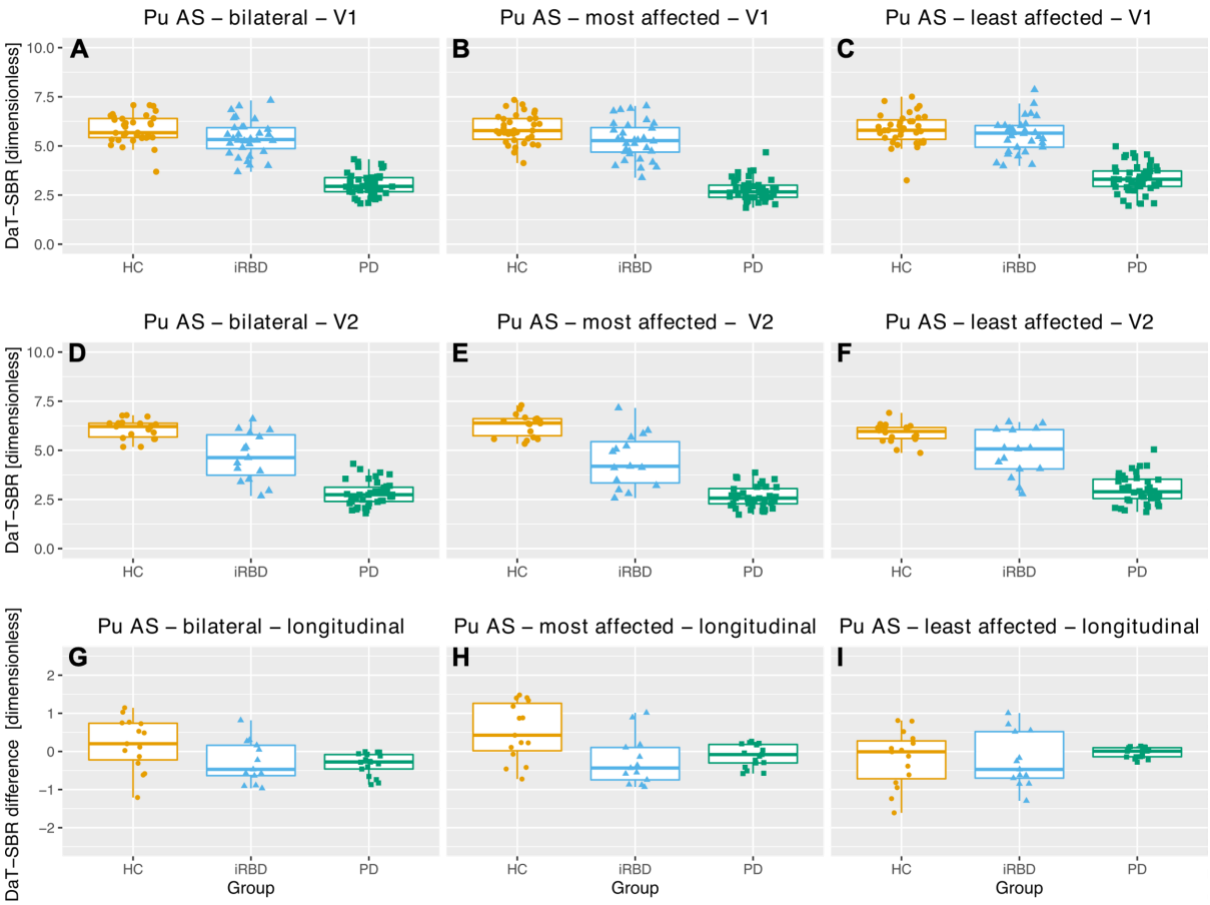
Supplementary Figure 3 Scatter boxplots of age and sex adjusted DaT-SBR measurements in the associative caudate nucleus. (A-C) DaT-SBR at V1. **(D-F)** DaT-SBR at V2. **(G-I)** DaT-SBR difference in longitudinal examinations (V2 – V1). The left column shows bilateral values. The centre and right columns respectively show values in the clinically most and least affected brain hemispheres
AS = associative; Cd = caudate nucleus; DaT = dopamine transporter; HC = healthy control; iRBD = idiopathic REM sleep behaviour disorder; PD = Parkinson's disease; SBR = striatal binding ratio; V1/V2 = visit 1/2



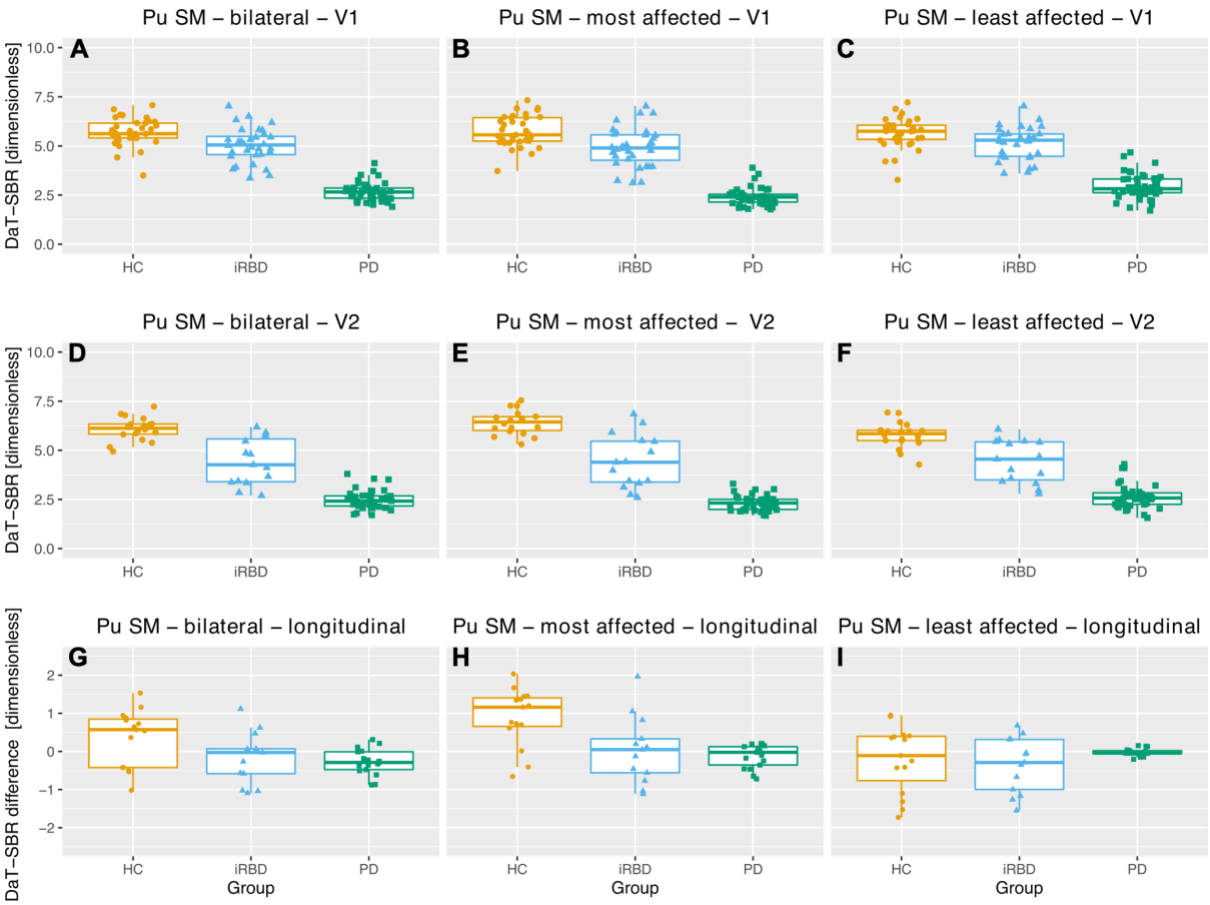
Supplementary Figure 4 Scatter boxplots of age and sex adjusted DaT-SBR measurements in the sensorimotor caudate nucleus. (A-C) DaT-SBR at V1. **(D-F)** DaT-SBR at V2. **(G-I)** DaT-SBR difference in longitudinal examinations (V2 – V1). The left column shows bilateral values. The centre and right columns respectively show values in the clinically most and least affected brain hemispheres
Cd = caudate nucleus; DaT = dopamine transporter; HC = healthy control; iRBD = idiopathic REM sleep behaviour disorder; PD = Parkinson's disease; SBR = striatal binding ratio; SM = sensorimotor; V1/V2 = visit 1/2



Supplementary Figure 5 Scatter boxplots of age and sex adjusted DaT-SBR measurements in the limbic putamen. (A-C) DaT-SBR at V1. **(D-F)** DaT-SBR at V2. **(G-I)** DaT-SBR difference in longitudinal examinations (V2 – V1). The left column shows bilateral values. The centre and right columns respectively show values in the clinically most and least affected brain hemispheres
DaT = dopamine transporter; HC = healthy control; iRBD = idiopathic REM sleep behaviour disorder; LI = limbic; PD = Parkinson's disease; Pu = putamen; SBR = striatal binding ratio; V1/V2 = visit 1/2

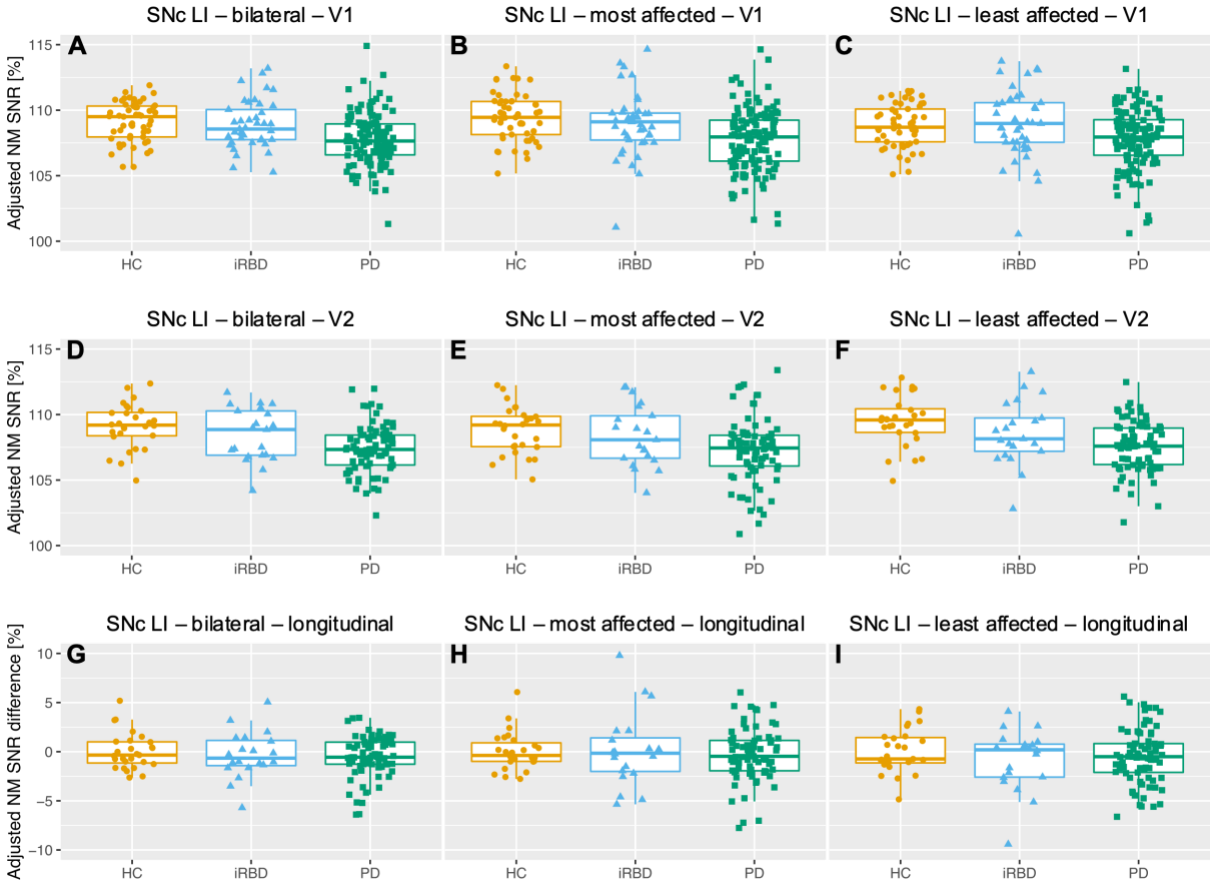


Supplementary Figure 6 Scatter boxplots of age and sex adjusted DaT-SBR measurements in the associative putamen. **(A-C)** DaT-SBR at V1. **(D-F)** DaT-SBR at V2. **(G-I)** DaT-SBR difference in longitudinal examinations (V2 – V1). The left column shows bilateral values. The centre and right columns respectively show values in the clinically most and least affected brain hemispheres
AS = associative; DaT = dopamine transporter; HC = healthy control; iRBD = idiopathic REM sleep behaviour disorder; PD = Parkinson's disease; Pu = putamen; SBR = striatal binding ratio; V1/V2 = visit 1/2

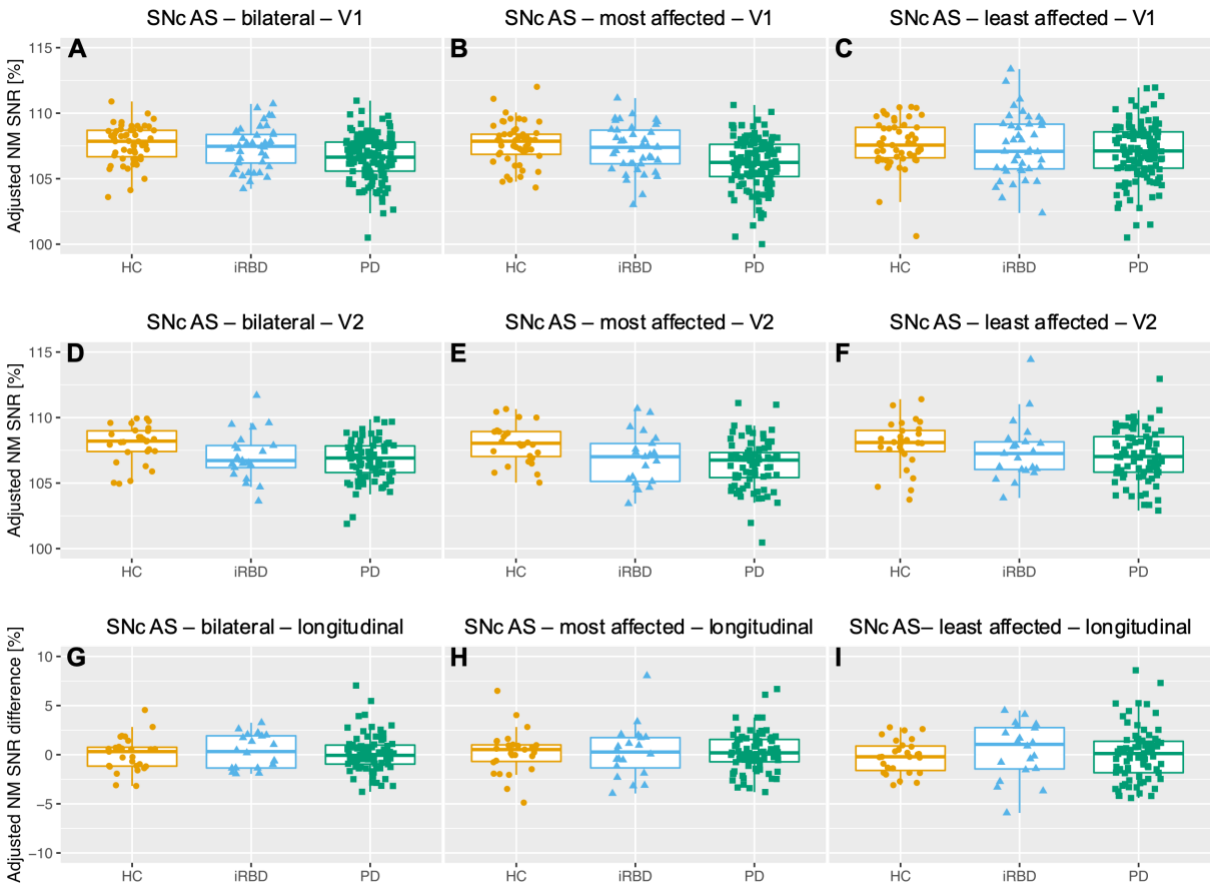


Supplementary Figure 7 Scatter boxplots of age and sex adjusted DaT-SBR measurements in the sensorimotor putamen. (A-C) DaT-SBR at V1. **(D-F)** DaT-SBR at V2. **(G-I)** DaT-SBR difference in longitudinal examinations (V2 – V1). The left column shows bilateral values. The centre and right columns respectively show values in the clinically most and least affected brain hemispheres

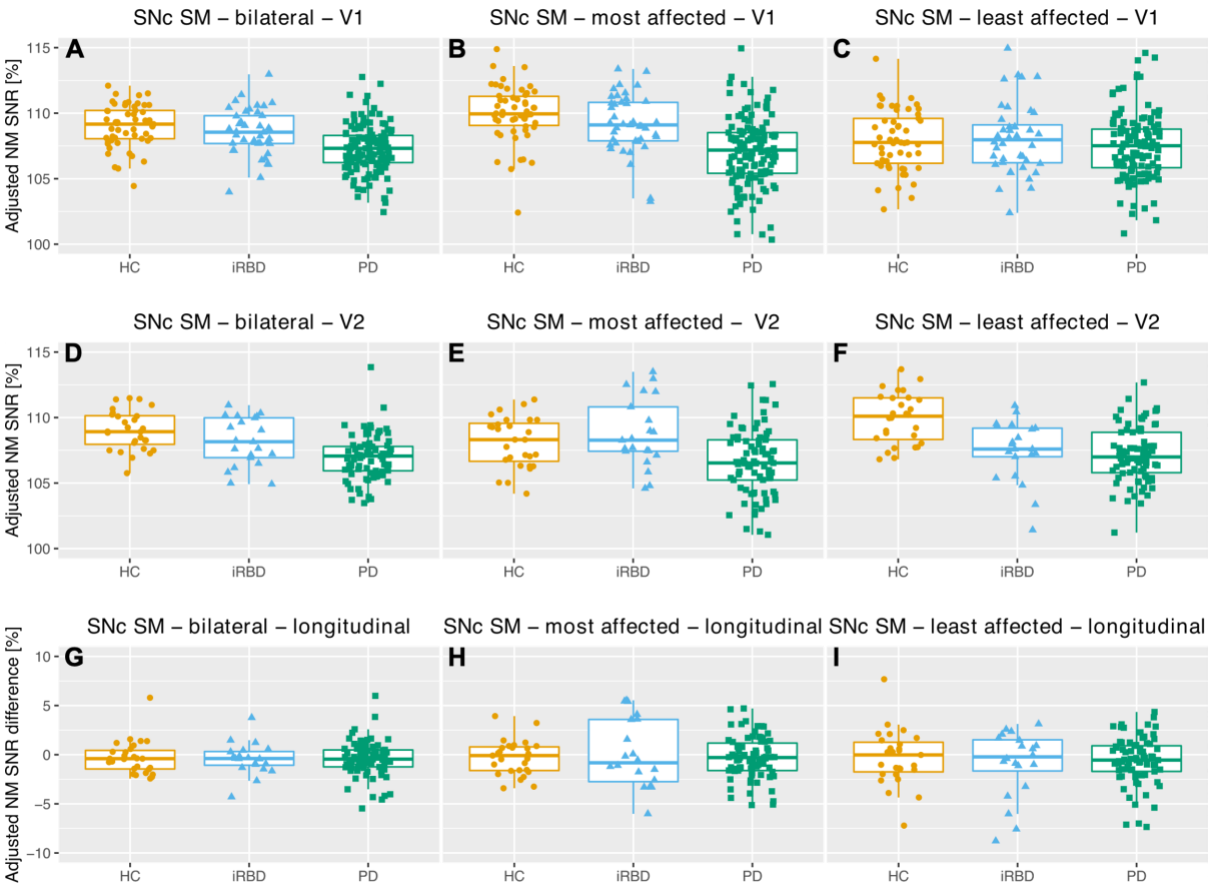
DaT = dopamine transporter; HC = healthy control; iRBD = idiopathic REM sleep behaviour disorder; PD = Parkinson's disease; Pu = putamen; SBR = striatal binding ratio; SM = sensorimotor; V1/V2 = visit 1/2



Supplementary Figure 8 Scatter boxplots of age and sex adjusted neuromelanin SNR measurements in the limbic SNc. **(A-C)** NM SNR at V1. **(D-F)** NM SNR at V2. **(G-I)** NM SNR difference in longitudinal examinations (V2 – V1). The left column shows bilateral values. The centre and right columns respectively show values in the clinically most and least affected brain hemispheres
HC = healthy control; IRBD = idiopathic REM sleep behaviour disorder; LI = limbic; NM = neuromelanin; PD = Parkinson's disease; SNc = substantia nigra pars compacta; SNR = signal-to-noise ratio; V1/V2 = visit 1/2

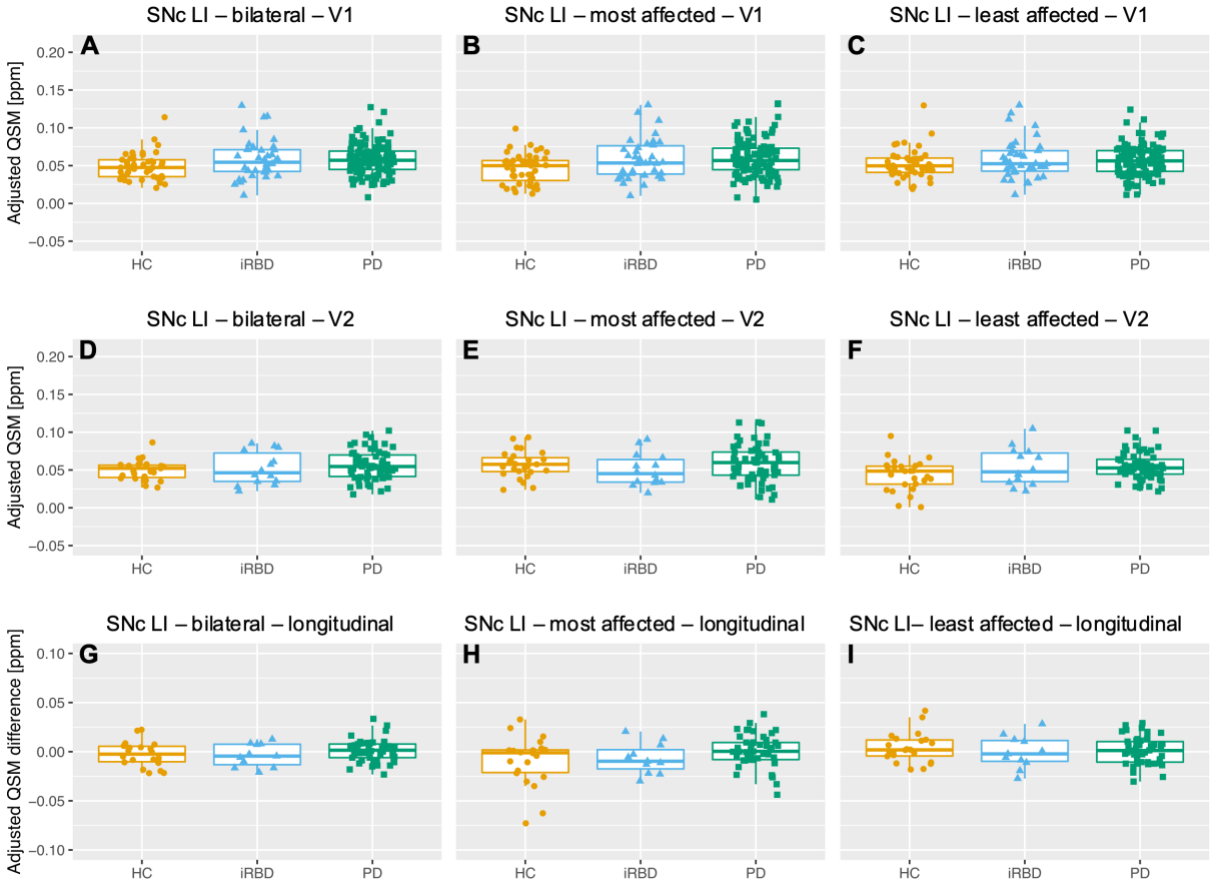


Supplementary Figure 9 Scatter boxplots of age and sex adjusted neuromelanin SNR measurements in the associative SNc. (A-C) NM SNR at V1. **(D-F)** NM SNR at V2. **(G-I)** NM SNR difference in longitudinal examinations (V2 – V1). The left column shows bilateral values. The centre and right columns respectively show values in the clinically most and least affected brain hemispheres AS = associative; HC = healthy control; IRBD = idiopathic REM sleep behaviour disorder; NM = neuromelanin; PD = Parkinson's disease; SNc = substantia nigra pars compacta; SNR = signal-to-noise ratio; V1/V2 = visit 1/2

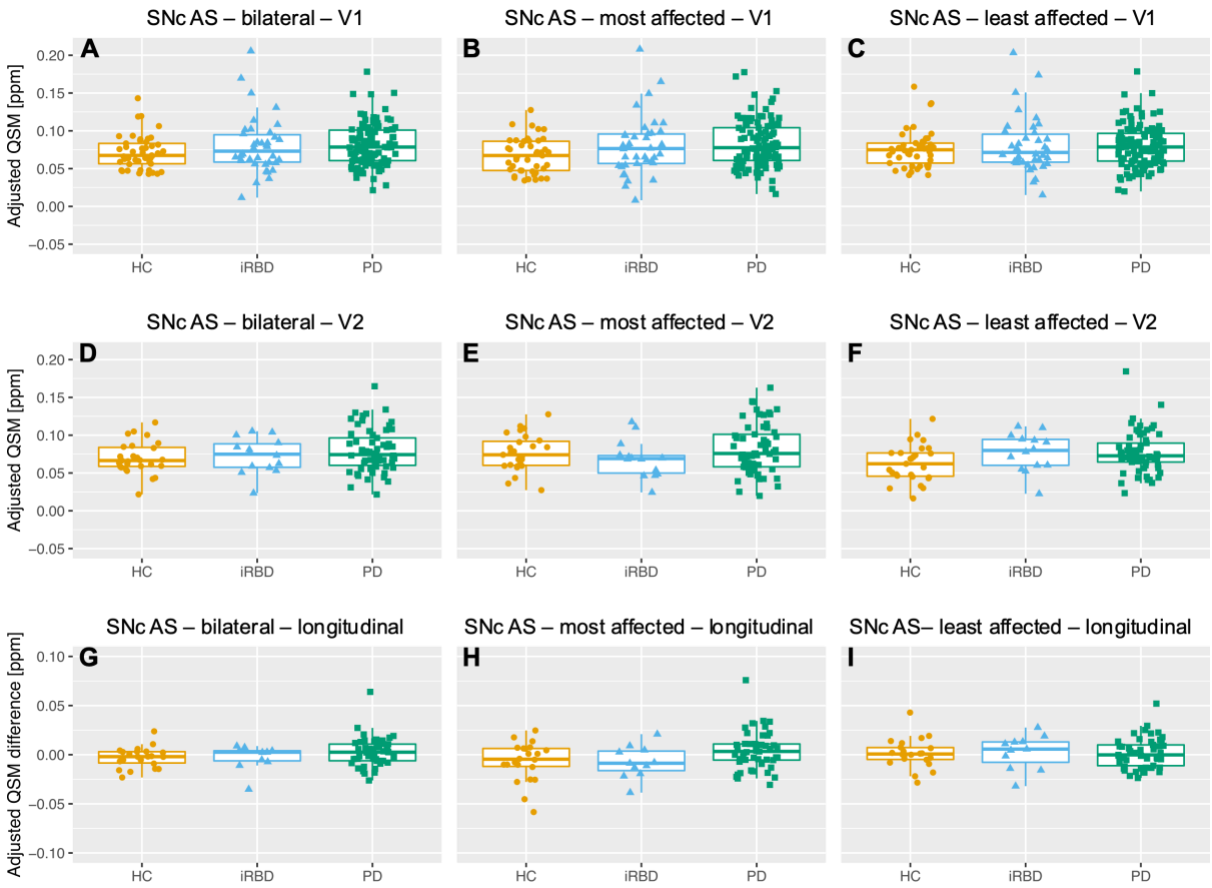


Supplementary Figure 10 Scatter boxplots of age and sex adjusted neuromelanin SNR measurements in the sensorimotor SNc. (A-C) NM SNR at V1. **(D-F)** NM SNR at V2. **(G-I)** NM SNR difference in longitudinal examinations (V2 – V1). The left column shows bilateral values. The centre and right columns respectively show values in the clinically most and least affected brain hemispheres

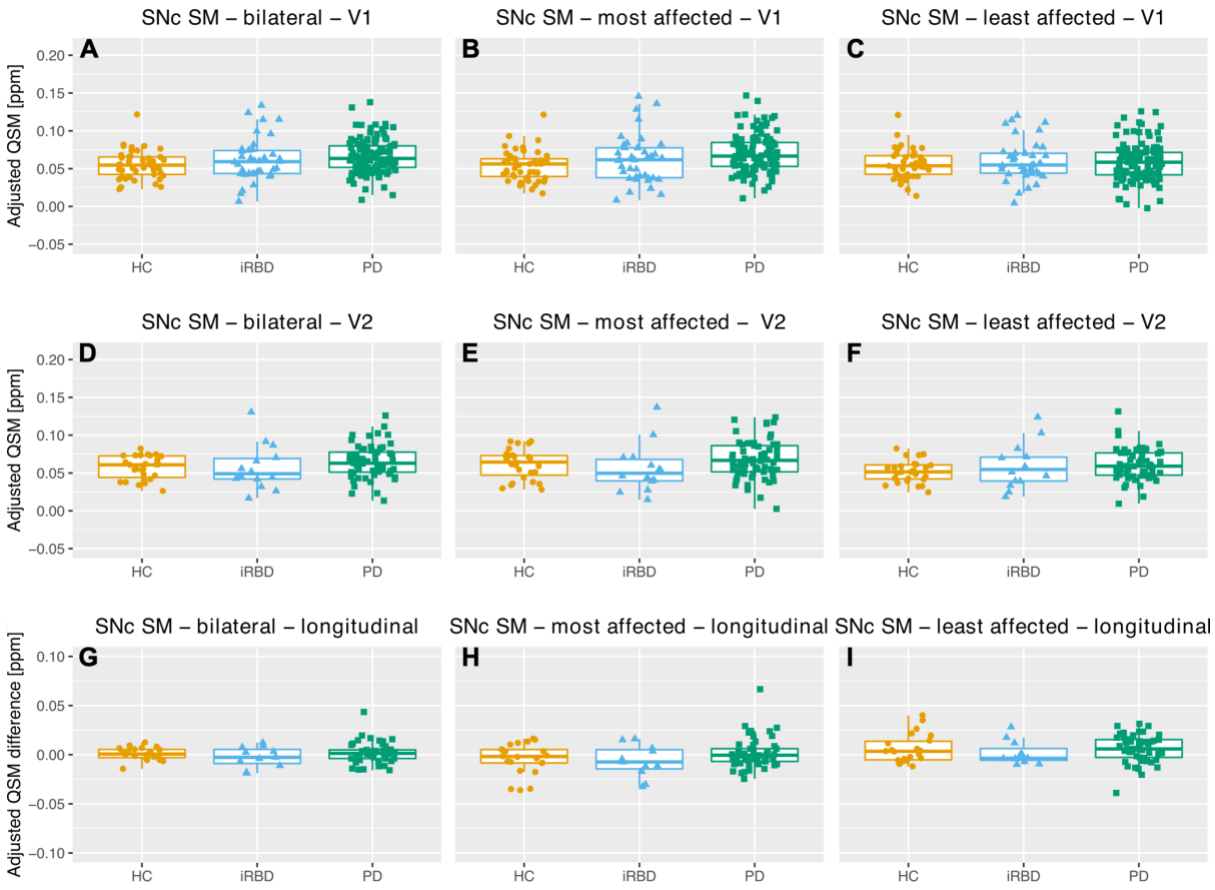
HC = healthy control; IRBD = idiopathic REM sleep behaviour disorder; NM = neuromelanin; PD = Parkinson's disease; SM = sensorimotor; SNc = substantia nigra pars compacta; SNR = signal-to-noise ratio; V1/V2 = visit ½



Supplementary Figure 11 Scatter boxplots of age and sex adjusted QSM measurements in the limbic SNc. (A-C) QSM at V1. **(D-F)** QSM at V2. **(G-I)** QSM difference in longitudinal examinations (V2 – V1). The left column shows bilateral values. The centre and right columns respectively show values in the clinically most and least affected brain hemispheres
HC = healthy control; IRBD = idiopathic REM sleep behaviour disorder; LI = limbic; PD = Parkinson's disease; ppm = parts per million; QSM = quantitative susceptibility mapping; SNc = substantia nigra pars compacta; V1/V2 = visit 1/2



Supplementary Figure 12 Scatter boxplots of age and sex adjusted QSM measurements in the associative SNc. (A-C) QSM at V1. **(D-F)** QSM at V2. **(G-I)** QSM difference in longitudinal examinations (V2 – V1). The left column shows bilateral values. The centre and right columns respectively show values in the clinically most and least affected brain hemispheres
AS = associative; HC = healthy control; IRBD = idiopathic REM sleep behaviour disorder; PD = Parkinson's disease; ppm = parts per million; QSM = quantitative susceptibility mapping; SNc = substantia nigra pars compacta; V1/V2 = visit 1/2



Supplementary Figure 13 Scatter boxplots of age and sex adjusted QSM measurements in the sensorimotor SNc. (A-C) QSM at V1. **(D-F)** QSM at V2. **(G-I)** QSM difference in longitudinal examinations (V2 – V1). The left column shows bilateral values. The centre and right columns respectively show values in the clinically most and least affected brain hemispheres
HC = healthy control; IRBD = idiopathic REM sleep behaviour disorder; PD = Parkinson's disease; ppm = parts per million; SM = sensorimotor; QSM = quantitative susceptibility mapping; SNc = substantia nigra pars compacta; V1/V2 = visit 1/2

References

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